U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		ATTORNEY'S DOCKET NUMBER
TRANMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		UEMURA 7
		U.S. APPLICATION NO. (If known,see 37 CFR 1.5) 09/856371
INTERNATIONAL APPLICATION NO. PCT/JP99/06475	INTERNATIONAL FILING DATE 19 November 1999	PRIORITY CLAIMED 20 November 1998

TITLE OF INVENTION
NOVEL SERINE PROTEASE BSSP2

APPLICANT(S) FOR DO/EO/US
Hidetoshi UEMURA et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [X] This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. [X] The US has been elected in a Demand by the expiration of 19 months from the priority date (PCT Article 31).
5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [ ] is attached hereto (required only if not transmitted by the International Bureau).
   b. [X] has been communicated by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [X] An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
7. [X] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [ ] are transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] have been communicated by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [X] have not been made and will not be made.
8. [ ] An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. [X] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. [ ] An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. [ ] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An Assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [X] A FIRST preliminary amendment.
    [ ] A SECOND or SUBSEQUENT preliminary amendment.
14. [ ] A substitute specification.
15. [ ] A change of power of attorney and/or address letter.
16. [X] Other items or information:
    [X] Courtesy copy of the first page of the International Publication (WO 00/31272).
    [X] Courtesy copy of the International Preliminary Examination Report (In Japanese).
    [X] Formal drawings, 07 sheets, Figures 1-7.
    [X] Courtesy Copy of the International Search Report.
    [X] Application Data Sheet
    [X] The application is (or will be) assigned to: FUSO PHARMACEUTICAL INDUSTRIES, LTD. whose address is 7-10, Doshomachi 1-chome, Chuo-ku, Osaka-shi, Osaka, Japan.

PCT09

RAW SEQUENCE LISTING DATE: 10/11/2001
PATENT APPLICATION: **US/09/856,371** TIME: 09:51:46

Input Set : **A:\sequence listing.txt**
Output Set: **N:\CRF3\10112001\I856371.raw**

<110> APPLICANT: UEMURA, Hidetoshi
OKUI, Akira
KOMINAMI, Katsuya
YAMAGUCHI, Nozomi
MITSUI, Shinichi

<120> TITLE OF INVENTION: NOVEL SERINE PROTEASE BSSP2

<130> FILE REFERENCE: UEMURA=7

<140> CURRENT APPLICATION NUMBER: 09/856,371
<141> CURRENT FILING DATE: 2001-05-21

<150> PRIOR APPLICATION NUMBER: JP 10/347785
<151> PRIOR FILING DATE: 1998-11-20

<150> PRIOR APPLICATION NUMBER: PCT/JP99/06475
<151> PRIOR FILING DATE: 1999-11-19

<160> NUMBER OF SEQ ID NOS: 44

<170> SOFTWARE: PatentIn version 3.1

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            20                  25                  30

cca cac tgg gta gtg act gct gcc cac tgc atg tac agt ttc agg ctg      144
Pro His Trp Val Val Thr Ala Ala His Cys Met Tyr Ser Phe Arg Leu
        35                  40                  45

tcc cgc cta tcc agc tgg cgg gtt cat gca ggg ctg gtc agc cat ggt      192
Ser Arg Leu Ser Ser Trp Arg Val His Ala Gly Leu Val Ser His Gly
    50                  55                  60

gct gtc cga caa cac cag gga act atg gtg gag aag atc att cct cat      240
Ala Val Arg Gln His Gln Gly Thr Met Val Glu Lys Ile Ile Pro His
65                  70                  75                  80

cct ttg tac agt gcc cag aac cat gac tat gat gtg gct ctg ctg cag      288
Pro Leu Tyr Ser Ala Gln Asn His Asp Tyr Asp Val Ala Leu Leu Gln
                85                  90                  95

ctc cgg aca cca atc aac ttc tca gac acc gtg gac gct gtg tgc ttg      336
Leu Arg Thr Pro Ile Asn Phe Ser Asp Thr Val Asp Ala Val Cys Leu
            100                 105                 110

ccg gcc aag gag cag tac ttt cca tgg ggg tcg cag tgc tgg gtg tct      384
Pro Ala Lys Glu Gln Tyr Phe Pro Trp Gly Ser Gln Cys Trp Val Ser
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ENTERED

P.5

RAW SEQUENCE LISTING DATE: 10/11/2001
PATENT APPLICATION: US/09/856,371 TIME: 09:51:46

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Output Set: N:\CRF3\10112001\I856371.raw

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 74 cag gac aca atg gta ccc ctg ctc agc acc cac ctc tgc aac agc tca      480
 75 Gln Asp Thr Met Val Pro Leu Leu Ser Thr His Leu Cys Asn Ser Ser
 76 145                 150                 155                 160
 78 tgc atg tac agt ggg gca ctt aca cac cgc atg ttg tgt gct ggc tac      528
 79 Cys Met Tyr Ser Gly Ala Leu Thr His Arg Met Leu Cys Ala Gly Tyr
 80                 165                 170                 175
 82 ctg gat gga agg gca gac gca tgc cag gga gac agc ggg gga ccc ctg      576
 83 Leu Asp Gly Arg Ala Asp Ala Cys Gln Gly Asp Ser Gly Gly Pro Leu
 84             180                 185                 190
 86 gta tgt ccc agt ggt gac acg tgg cac ctt gta ggg gtg gtc agc tgg      624
 87 Val Cys Pro Ser Gly Asp Thr Trp His Leu Val Gly Val Val Ser Trp
 88         195                 200                 205
 90 ggt cgt ggc tgt gca gag ccc aat cgc cca ggt gtc tat gcc aag gta      672
 91 Gly Arg Gly Cys Ala Glu Pro Asn Arg Pro Gly Val Tyr Ala Lys Val
 92     210                 215                 220
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 96 225                 230                 235
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102 <213> ORGANISM: Mus sp.
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111             20                  25                  30
114 Pro His Trp Val Val Thr Ala Ala His Cys Met Tyr Ser Phe Arg Leu
115         35                  40                  45
118 Ser Arg Leu Ser Ser Trp Arg Val His Ala Gly Leu Val Ser His Gly
119     50                  55                  60
122 Ala Val Arg Gln His Gln Gly Thr Met Val Glu Lys Ile Ile Pro His
123 65                  70                  75                  80
126 Pro Leu Tyr Ser Ala Gln Asn His Asp Tyr Asp Val Ala Leu Leu Gln
127                 85                  90                  95
130 Leu Arg Thr Pro Ile Asn Phe Ser Asp Thr Val Asp Ala Val Cys Leu
131             100                 105                 110
134 Pro Ala Lys Glu Gln Tyr Phe Pro Trp Gly Ser Gln Cys Trp Val Ser
135         115                 120                 125
138 Gly Trp Gly His Thr Asp Pro Ser His Thr His Ser Ser Asp Thr Leu
139     130                 135                 140
142 Gln Asp Thr Met Val Pro Leu Leu Ser Thr His Leu Cys Asn Ser Ser
143 145                 150                 155                 160
146 Cys Met Tyr Ser Gly Ala Leu Thr His Arg Met Leu Cys Ala Gly Tyr
147                 165                 170                 175
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RAW SEQUENCE LISTING DATE: 10/11/2001
PATENT APPLICATION: US/09/856,371 TIME: 09:51:46

Input Set : A:\sequence listing.txt
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151             180                 185                 190
154 Val Cys Pro Ser Gly Asp Thr Trp His Leu Val Gly Val Val Ser Trp
155         195                 200                 205
158 Gly Arg Gly Cys Ala Glu Pro Asn Arg Pro Gly Val Tyr Ala Lys Val
159     210                 215                 220
162 Ala Glu Phe Leu Asp Trp Ile His Asp Thr Val Gln Val Arg
163 225                 230                 235
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167 <211> LENGTH: 1685
168 <212> TYPE: DNA
169 <213> ORGANISM: Mus sp.
171 <220> FEATURE:
172 <221> NAME/KEY: CDS
173 <222> LOCATION: (247)..(1065)
174 <223> OTHER INFORMATION:
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180 tcggccagac tggctcctgg tatgccatga gggccggagc ccagccctgg gcatgcacat    120
182 ctgcaagagt cttgggcata tcaggcttac tcaacacaag gccgtgaatc tgtctgacat    180
184 caagctcaac agatcccagg agtttgctca actctctgct agaccgggag gccttgtaga    240
186 ggaggc atg gaa gcc cag gta ggg ctt ctg tgg gtt agc gct aac tgt       288
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188         1               5                   10
190 cct tct ggc cga att gtt tct ctc aaa tgt tct gag tgt ggg gca agg      336
191 Pro Ser Gly Arg Ile Val Ser Leu Lys Cys Ser Glu Cys Gly Ala Arg
192 15                  20                  25                  30
194 cct ctg gct tct cga ata gtt ggc ggc caa gct gtg gct tct ggg cgc      384
195 Pro Leu Ala Ser Arg Ile Val Gly Gly Gln Ala Val Ala Ser Gly Arg
196                 35                  40                  45
198 tgg cca tgg caa gct agc gtg atg ctt ggc tcc cgg cac acg tgt ggg      432
199 Trp Pro Trp Gln Ala Ser Val Met Leu Gly Ser Arg His Thr Cys Gly
200             50                  55                  60
202 gcc tct gtg ttg gca cca cac tgg gta gtg act gct gcc cac tgc atg      480
203 Ala Ser Val Leu Ala Pro His Trp Val Val Thr Ala Ala His Cys Met
204         65                  70                  75
206 tac agt ttc agg ctg tcc cgc cta tcc agc tgg cgg gtt cat gca ggg      528
207 Tyr Ser Phe Arg Leu Ser Arg Leu Ser Ser Trp Arg Val His Ala Gly
208     80                  85                  90
210 ctg gtc agc cat ggt gct gtc cga caa cac cag gga act atg gtg gag      576
211 Leu Val Ser His Gly Ala Val Arg Gln His Gln Gly Thr Met Val Glu
212 95                  100                 105                 110
214 aag atc att cct cat cct ttg tac agt gcc cag aac cat gac tat gat      624
215 Lys Ile Ile Pro His Pro Leu Tyr Ser Ala Gln Asn His Asp Tyr Asp
216                 115                 120                 125
218 gtg gct ctg ctg cag ctc cgg aca cca atc aac ttc tca gac acc gtg      672
219 Val Ala Leu Leu Gln Leu Arg Thr Pro Ile Asn Phe Ser Asp Thr Val
220             130                 135                 140
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RAW SEQUENCE LISTING DATE: 10/11/2001
PATENT APPLICATION: **US/09/856,371** TIME: 09:51:46

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230 agc tca gat aca ctg cag gac aca atg gta ccc ctg ctc agc acc cac      816
231 Ser Ser Asp Thr Leu Gln Asp Thr Met Val Pro Leu Leu Ser Thr His
232 175                 180                 185                 190
234 ctc tgc aac agc tca tgc atg tac agt ggg gca ctt aca cac cgc atg      864
235 Leu Cys Asn Ser Ser Cys Met Tyr Ser Gly Ala Leu Thr His Arg Met
236                 195                 200                 205
238 ttg tgt gct ggc tac ctg gat gga agg gca gac gca tgc cag gga gac      912
239 Leu Cys Ala Gly Tyr Leu Asp Gly Arg Ala Asp Ala Cys Gln Gly Asp
240             210                 215                 220
242 agc ggg gga ccc ctg gta tgt ccc agt ggt gac acg tgg cac ctt gta      960
243 Ser Gly Gly Pro Leu Val Cys Pro Ser Gly Asp Thr Trp His Leu Val
244         225                 230                 235
246 ggg gtg gtc agc tgg ggt cgt ggc tgt gca gag ccc aat cgc cca ggt     1008
247 Gly Val Val Ser Trp Gly Arg Gly Cys Ala Glu Pro Asn Arg Pro Gly
248     240                 245                 250
250 gtc tat gcc aag gta gca gag ttc ctg gac tgg atc cat gac act gtg     1056
251 Val Tyr Ala Lys Val Ala Glu Phe Leu Asp Trp Ile His Asp Thr Val
252 255                 260                 265                 270
254 cag gtc cgc tagccgaaga agcagcagca gccacctgtg acgccgagct             1105
255 Gln Val Arg
258 gtggatcgcc catggatcac cccagtctgg gggccagcat ctgggtcact gggcctctcc   1165
260 ccaaaggctc tgacttcgag ttcatctttc tcatctgaga acctccacaa caggaaaagg   1225
262 agtctgcggc tagattggga atgatggtga gaggaaggga taggaggaca gaagagacag   1285
264 cagaggcttc tggaagcatc tgggagactg ctcctctgct ccccccacac cccacgtgca   1345
266 tccactgggg gatgctggag atgcccaatc cttgtttctt gtggggccac tggaaggcta   1405
268 agtccaactt tagaggatgc cctgtctcga gagttactag gcagataagg ttaaggttgg   1465
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272 aggtaagcta atagccccgc accaggcaga ggtctacagg gtaagaagga tgcagttggg   1585
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294 Ala Ser Arg Ile Val Gly Gly Gln Ala Val Ala Ser Gly Arg Trp Pro
295         35                  40                  45
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299     50                  55                  60
302 Val Leu Ala Pro His Trp Val Val Thr Ala Ala His Cys Met Tyr Ser
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RAW SEQUENCE LISTING DATE: 10/11/2001
PATENT APPLICATION: **US/09/856,371** TIME: 09:51:46

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311             100                 105                 110
314 Ile Pro His Pro Leu Tyr Ser Ala Gln Asn His Asp Tyr Asp Val Ala
315         115                 120                 125
318 Leu Leu Gln Leu Arg Thr Pro Ile Asn Phe Ser Asp Thr Val Asp Ala
319     130                 135                 140
322 Val Cys Leu Pro Ala Lys Glu Gln Tyr Phe Pro Trp Gly Ser Gln Cys
323 145                 150                 155                 160
326 Trp Val Ser Gly Trp Gly His Thr Asp Pro Ser His Thr His Ser Ser
327                 165                 170                 175
330 Asp Thr Leu Gln Asp Thr Met Val Pro Leu Leu Ser Thr His Leu Cys
331             180                 185                 190
334 Asn Ser Ser Cys Met Tyr Ser Gly Ala Leu Thr His Arg Met Leu Cys
335         195                 200                 205
338 Ala Gly Tyr Leu Asp Gly Arg Ala Asp Ala Cys Gln Gly Asp Ser Gly
339     210                 215                 220
342 Gly Pro Leu Val Cys Pro Ser Gly Asp Thr Trp His Leu Val Gly Val
343 225                 230                 235                 240
346 Val Ser Trp Gly Arg Gly Cys Ala Glu Pro Asn Arg Pro Gly Val Tyr
347                 245                 250                 255
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374 ccaatggaga ttcggtgcac ggaagagggt gctgggcctg ggatcttcag aatggagttg    180
376 ggagaccaga ggcaatccat ttctcagtcc caacgctggt gctgcctgca acgtggctgt    240
378 gtaatactgg gcgtcctggg gctgctggct ggagcaggca ttgcttcatg gctcttagtg    300
380 ttgtatctat ggccggctgc ctctccatcc atctctggga cgttgcagga ggaggagatg    360
382 actttgaact gtccaggagt gagctgtgag gaagagctcc ttccatctct tcccaaaaca    420
384 gaataaatgg aggggatctt ctgcttcaag tacaagtaag agctcggcca gactggctcc    480
386 tggtctgcca tgagggctgg agccccgccc tgggc atg cac atc tgc aag agt      533
387                                       Met His Ile Cys Lys Ser
388                                       1               5
390 ctt ggg cat atc agg ctt act caa cac aag gcc gtg aat ctg tct gac      581
391 Leu Gly His Ile Arg Leu Thr Gln His Lys Ala Val Asn Leu Ser Asp
392             10                  15                  20
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Use of n and / or Xaa has been detected in the Sequence Listing. Review the Sequence Listing to ensure a corresponding explanation is present in the <220> to <223> fields of each sequence using n or Xaa.

VERIFICATION SUMMARY DATE: 10/11/2001
PATENT APPLICATION: **US/09/856,371** TIME: 09:51:47

Input Set : **A:\sequence listing.txt**
Output Set: **N:\CRF3\10112001\I856371.raw**

L:1325 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:20
L:1355 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:21

#4

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

ATTY.'S DOCKET: UEMURA7

In re Application of:	)	Art Unit:
	)	
H. UEMURA, et al.	)	Examiner:
	)	
Serial No.: 09/856,371	)	Confirmation No.
	)	
Filed: May 21, 2001	)	Washington D.C.
	)	
For: NOVEL SERINE PROTEASE BSSP2	)	September 4, 2001
	)	

RESPONSE TO NOTIFICATION TO COMPLY WITH REQUIREMENTS FOR PATENT APPLICATIONS CONTAINING NUCLEOTIDE SEQUENCE AND/OR AMINO ACID SEQUENCE DISCLOSURES

Honorable Commissioner for Patents
Washington, D.C. 20231

In response to the Notice to Comply, dated July 3, 2001, and prior to the examination of the above-described application, please amend the present application as follows:

IN THE SPECIFICATION

Please replace the paragraph beginning at page 19, line 10, with the following rewritten paragraph:

--The novel mouse serine protease can be divided into types 1, 2 and 3. It has been shown that type 1 is composed of 273 amino acids, type 2 is composed of 311 amino acids, and type 3 is composed of 445 amino acids. These amino acid sequences contain a common amino acid sequence of 238 amino acids whose N-terminus side starts with Ile-Val-Gly-Gly-Gln-Ala-Val (amino acid

1-7 of SEQ ID NO:2) as the mature serine protease. Further, the amino acid sequence of the mature serine protease contains a consensus sequence having serine protease activity. Since there are two or more amino acid sequences which are characteristic of sugar chain binding sites, the amino acid sequence is presumed to have at least two sugar chains.--

Please replace the paragraph beginning at the bottom of page 26, line 14, with the following rewritten paragraph:

--The vector is not specifically limited in so far as it can express the protein of the present invention. Examples thereof include pBAD/His, pRSETA, pcDNA2.1, pTrcHis2A, pYES2, pBlueBac4.5, pcDNA3.1 and pSecTag2 manufacture by Invitrogen, pET and pBAC manufactured by Novagen, pGEM manufactured by Promega, pBluescriptII manufactured by Stratagene, pGEX and pUC18/19 manufactured by Pharmacia, PfastBAC1 manufactured by GIBCO and the like. Preferably, a protein expression vector (described in the specification of a patent application entitled "Protein expression vector and its use" and filed by the same applicant on the same day) is used. This expression vector is constructed by using pCRII-TOPO vector described in the Examples hereinafter, or a commercially available expression vector, for example pSecTag2A vector or pSecTag2B vector (Invitrogen) and integrating a secretory signal nucleotide sequence suitable for expression of the protein of the present invention, in the 3' downstream side

thereof, a Tag nucleotide sequence, a cleavable nucleotide sequence and a cloning site, into which a nucleotide sequence encoding a target protein can be inserted, in this order. More specifically, it is preferred to use trypsin signal as the secretory signal, a nucleotide sequence encoding polyhistidine as the Tag nucleotide sequence, and a nucleotide sequence encoding an amino acid sequence which is susceptible to enzyme-specific cleavage, i.e., a nucleotide sequence encoding the amino acid sequence of Asp-Asp-Asp-Asp-Lys SEQ ID NO:42 (said amino acid sequence is recognized by enterokinase, and the recombinant fusion protein is cleaved at the C-terminus part thereof) as the cleavable nucleotide sequence.--

Please replace the paragraph beginning at page 49, line 22, with the following rewritten paragraph:

--The cloning was carried out by PCR using a mouse brain cDNA library (Clontech) as a template and nucleotide sequences corresponding to an amino acid sequence common to serine proteases represented by

Primer 1: GTG CTC ACN GCN GCB CAY TG (SEQ ID NO: 20)

Primer 2: CCV CTR WSD CCN CCN GGC GA (SEQ ID NO: 21)

as primers. Namely, 5 µl of the template, 5 µl of 10 x ExTaq buffer, 5 µl of dNTP, 10 pmol of each of the above primers and 0.5 µl of ExTaq (TAKARA) were added and the total volume was adjusted to 50 µl with sterilized water. PCR was carried out by

repeating a cycle of heating at 94°C for 0.5 minute, at 55°C for 0.5 minute and then at 72°C for 1 minute, 30 times. The PCR product was mixed with pCR II-TOPO vector attached to TOPO TA cloning kit (Invitrogen) and the mixture was allowed to stand at room temperature for 5 minutes. Then, according to a conventional manner, *E. coli* Top 10 attached to the kit was transformed and applied to a LB (Amp^+) plate (containing 100 µg/ml of ampicillin). According to a conventional manner, a plasmid was extracted from each colony obtained and its nucleotide sequence was determined by cycle sequencing method with a fluorescence sequencer (ABI). Homology of the sequence of each clone was examined by means of GenBank. Regarding an unknown sequence, i.e., BSSP2 gene, the full length cDNA was obtained by 5' RACE and 3' RACE and, according to the same manner as described above, the nucleotide sequence was determined. Namely, BSSP2 clone specific primers, GSP1 primers [mBSSP2.2 (SEQ ID NO: 27) or mBSSP2.0 (SEQ ID NO: 22)] and GSP2 primers [mBSSP2R2 (SEQ ID NO: 28) or mBSSP2.1 (SEQ ID NO: 23)] were prepared. PCR was carried out by using mouse brain Marathon-Ready cDNA (Clontech), AP1 primer attached to this reagent and either of the above GSP1 primers and heating at 94°C for 2 minutes once and repeating a cycle of heating at 94°C for 30 seconds, at 60°C for 30 seconds and then at 72°C for 30 seconds 35 times. Then, 5 µl of the PCR product diluted to 1/100, 5 µl of 10 x buffer, 5 µl of dNTP, 10 pmol of either of 10 µM of the

above GSP2 primer, 10 pmol of AP2 primer attached to the above reagent and 0.5 unit of ExTaq were admixed and adjusted to 50 µl with sterilized water. Then, according to the same manner as the above, PCR was carried out. The PCR product was cloned by the above TOPO TA cloning kit and sequenced to obtain the upstream and downstream regions of the above clone. At this time, as for a clone which seemed not to cover the full length of a protein, the specific primers shown hereinafter were prepared based on the newly found nucleotide sequence. Further, based on this sequence, the primers capable of amplifying ORF as shown hereinafter [mBSSPF7 (SEQ ID NO: 26), mBSSP2R/E (SEQ ID NO: 29)] were prepared and PCR carried out using mouse brain Marathon-ready cDNA as a template to confirm that these clones were identical. This was cloned into pCR II-TOPO vector attached to TOPO TA cloning kit to obtain the plasmid pCR II/mBSSP2 containing the full length cDNA clone. The nucleotide sequence of DNA contained in this plasmid is shown in SEQ ID NO: 7 and the amino acid sequence of mSSP2 protein deduced from the nucleotide sequence is shown in SEQ ID NO: 8. Further, two different types of clones were obtained. The nucleotide sequences of these DNA are shown in SEQ ID NOS: 3 and 5, respectively. The amino acid sequences of mBSSP2 proteins deduced from these nucleotide sequences are shown in SEQ ID NOS: 4 and 6. These novel proteases are divided into types 1, 2 and 3. Type 1 is composed of 273 amino acids, type 2 is composed of 311 amino acids and

type 3 is composed of 445 amino acids. These amino acid sequences contained the common amino acid sequence composed of 238 amino acids whose N-terminus side started with Ile-Val-Gly-Gly-Gln-Ala-Val (amino acid 1-7 of SEQ ID NO:2) as the mature serine protease. Further, in the amino acid sequence of the mature serine protease, a consensus sequence having a serine protease activity was contained. Furthermore, since there were two or more amino acid sequences specific for a sugar chain bonding site, it was presumed that the amino acid sequence had at least two sugar chains.--

Please replace the paragraph beginning at the bottom of page 56, line 24, with the following rewritten paragraph:

--Amplification was carried out by using the primers having the sequences represented by SEQ ID NOS: 15 and 16 so that the peptide of Leu-Val-His-Gly (SEQ ID NO:43) was present at the C-terminus of the part from trypsin signal to the enterokinase recognition site of pSecTrypHis/neurosin. This was inserted between NheI and HindIII sites of pSecTag2A to construct the plasmid pTrypSig.--

Please replace the paragraph beginning at page 61, line 3, with the following rewritten paragraph:

--Reverse transcription of 1 µg of mRNA of human fetus brain (Clontech) was carried out by using Superscript II (Gibco

BRL) and oligo dT-Not I primer (5' GGCCACGCGTCGACTAGTA $C(T)_{17}$ 3' SEQ ID NO:44) to obtain cDNA. By using this as a template, PCR was carried out with primes prepared from mBSSP2 nucleotide sequence and represented by SEQ ID NOS: 30 and 31 to obtain a cDNA fragment of hBSSP2. Namely, 5 µl of the template, 5 µl of 10 x ExTaq buffer (TAKARA), 5 µl of dNTPs, 10 pmol portions of the above primers and 0.5 µl of ExTaq (TAKARA) were adjusted to 50 µl with sterilized water and PCR was carried out by repeating a cycle of heating at 94°C for 0.5 minute, at 55°C for 0.5 minute and then at 72°C for 1 minute, 35 times. The PCR reactions described hereinafter were carried out according to the same manner as the above composition and conditions except the template and primers. The PCR product was mixed with pGEM-T Easy vector (Promega) and Takara Ligation Solution I (TAKARA) and the reaction was carried out at 16°C for 2 hours. Then, according to the same manner, *E. coli* JM109 was transformed and applied to a LB (Amp^+) plate. A plasmid was extracted from each colony formed according to a conventional manner and its nucleotide sequence was determined by dideoxy method. As for a clone having homology to mBSSP2, full length cDNA was obtained by 5' RACE and 3' RACE and its sequence was determined as described above. PCR was carried out by using the above cDNA as a template and primers having the sequences represented by SEQ ID NOS: 30 and 37. 3' RACE was carried out by PCR using a 1/100 dilution of the above PCR product as a template and primers having the sequences

represented by SEQ ID NOS: 32 and 37. As for 5' RACE, cDNA for RACE was prepared from human fetal brain mRNA (Clontech) by using Superscript II and SMART RACE cDNA amplification kit (Clontech). PCR of this cDNA was carried out by using a primer of 10 x Universal Primer Mix (attached to the kit) and a primer having the sequence represented by SEQ ID NO: 33. Further, PCR was carried out by using the 1/100 dilution of the latter PCR product, a template, Nested PCR Primer (attached to the kit) and a primer having the sequence represented by SEQ ID NO: 34. The finally obtained PCR product was subjected to TA cloning as described above and the nucleotide sequence was determined to obtain the upstream and downstream regions of the above clone. In addition, primers for amplifying the full length cDNA as represented by SEQ ID NOS: 35 and 36 were prepared based on the resultant nucleotide sequence and PCR was carried out by using the above synthetic cDNA as a template. This PCR product was cloned into pGEM-T Easy vector to obtain the plasmid pGEM-TE/hBSSP2 containing the full length cDNA clone. The DNA sequence contained in this plasmid is shown in SEQ ID NO: 9 and hBSSP2 protein deduced from the nucleotide sequence is shown in SEQ ID NO: 10.--

IN THE SEQUENCE LISTING

Please substitute the attached Sequence Listing for that originally filed.

REMARKS

Applicants have added into the present specification a substitute paper copy Sequence Listing section according to 37 C.F.R. §1.821(c) as new pages 1-28. Furthermore, attached hereto is a 3 1/2" disk containing the "Sequence Listing" in computer readable form in accordance with 37 C.F.R. §1.821(e).

Applicants have amended the specification to insert SEQ ID Nos, as supported in the present specification.

The following statement is provided to meet the requirements of 37 C.F.R. §1.825(a) and 1.825(b).

I hereby state, in accordance with 37 C.F.R. §1.825(a), that the amendments included in the substitute sheets of the sequence listing are believed to be supported in the application as filed and that the substitute sheets of the sequence listing are not believed to include new matter.

I hereby further state, in accordance with 37 C.F.R. §1.825(b), that the attached copy of the computer readable form is the same as the attached substitute paper copy of the sequence listing.

Under U.S. rules, each sequence must be classified in <213> as an "Artificial Sequence", a sequence of "Unknown" origin, or a sequence originating in a particular organism, identified by its scientific name.

Neither the rules nor the MPEP clarify the nature of the relationship which must exist between a listed sequence and

an organism for that organism to be identified as the origin of the sequence under <213>.

Hence, counsel may choose to identify a listed sequence as associated with a particular organism even though that sequence does not occur in nature by itself in that organism (it may be, e.g., an epitopic fragment of a naturally occurring protein, or a cDNA of a naturally occurring mRNA, or even a substitution mutant of a naturally occurring sequence). Hence, the identification of an organism in <213> should not be construed as an admission that the sequence *per se* occurs in nature in said organism.

Similarly, designation of a sequence as "artificial" should not be construed as a representation that the sequence has no association with any organism. For example, a primer or probe may be designated as "artificial" even though it is necessarily complementary to some target sequence, which may occur in nature. Or an "artificial" sequence may be a substitution mutant of a natural sequence, or a chimera of two or more natural sequences, or a cDNA (i.e., intron-free sequence) corresponding to an intron-containing gene, or otherwise a fragment of a natural sequence.

The Examiner should be able to judge the relationship of the enumerated sequences to natural sequences by giving full consideration to the specification, the art cited therein, any further art cited in an IDS, and the results of his or her

sequence search against a database containing known natural sequences.

Attached hereto is a marked-up version of the changes made to the specification by the current amendment. The attached page is captioned "Version with markings to show changes made".

Applicants submit that the present application contains patentable subject matter and therefore urge the examiner to pass the case to issuance.

If the examiner has any questions or comments concerning the above described application, the examiner is urged to contact the undersigned at the phone number below.

Respectfully submitted,

BROWDY AND NEIMARK, P.L.L.C.
Attorneys for Applicant(s)

By _____
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F:\,A\Aoyb\Uemura 7\PTO\notice to comply with seq.wpd

VERSION WITH MARKINGS TO SHOW THE CHANGES MADE

The paragraph beginning at page 19, line 10, has been amended as follows:

The novel mouse serine protease can be divided into types 1, 2 and 3. It has been shown that type 1 is composed of 273 amino acids, type 2 is composed of 311 amino acids, and type 3 is composed of 445 amino acids. These amino acid sequences contain a common amino acid sequence of 238 amino acids whose N-terminus side starts with Ile-Val-Gly-Gly-Gln-Ala-Val <u>(amino acid 1-7 of SEQ ID NO:2)</u> as the mature serine protease. Further, the amino acid sequence of the mature serine protease contains a consensus sequence having serine protease activity. Since there are two or more amino acid sequences which are characteristic of sugar chain binding sites, the amino acid sequence is presumed to have at least two sugar chains.

The paragraph beginning at the bottom of page 26, line 14, has been amended as follows:

The vector is not specifically limited in so far as it can express the protein of the present invention. Examples thereof include pBAD/His, pRSETA, pcDNA2.1, pTrcHis2A, pYES2, pBlueBac4.5, pcDNA3.1 and pSecTag2 manufacture by Invitrogen, pET and pBAC manufactured by Novagen, pGEM manufactured by Promega, pBluescriptII manufactured by Stratagene, pGEX and pUC18/19 manufactured by Pharmacia, PfastBAC1 manufactured by GIBCO and the

like. Preferably, a protein expression vector (described in the specification of a patent application entitled "Protein expression vector and its use" and filed by the same applicant on the same day) is used. This expression vector is constructed by using pCRII-TOPO vector described in the Examples hereinafter, or a commercially available expression vector, for example pSecTag2A vector or pSecTag2B vector (Invitrogen) and integrating a secretory signal nucleotide sequence suitable for expression of the protein of the present invention, in the 3' downstream side thereof, a Tag nucleotide sequence, a cleavable nucleotide sequence and a cloning site, into which a nucleotide sequence encoding a target protein can be inserted, in this order. More specifically, it is preferred to use trypsin signal as the secretory signal, a nucleotide sequence encoding polyhistidine as the Tag nucleotide sequence, and a nucleotide sequence encoding an amino acid sequence which is susceptible to enzyme-specific cleavage, i.e., a nucleotide sequence encoding the amino acid sequence of Asp-Asp-Asp-Asp-Lys SEQ ID NO:42 (said amino acid sequence is recognized by enterokinase, and the recombinant fusion protein is cleaved at the C-terminus part thereof) as the cleavable nucleotide sequence.

The paragraph beginning at page 49, line 22, has been amended as follows:

The cloning was carried out by PCR using a mouse brain cDNA library (Clontech) as a template and nucleotide sequences corresponding to an amino acid sequence common to serine proteases represented by

Primer 1: GTG CTC ACN GCN GCB CAY TG (SEQ ID NO: 20)

Primer 2: CCV CTR WSD CCN CCN GGC GA (SEQ ID NO: 21)

as primers. Namely, 5 µl of the template, 5 µl of 10 x ExTaq buffer, 5 µl of dNTP, 10 pmol of each of the above primers and 0.5 µl of ExTaq (TAKARA) were added and the total volume was adjusted to 50 µl with sterilized water. PCR was carried out by repeating a cycle of heating at 94°C for 0.5 minute, at 55°C for 0.5 minute and then at 72°C for 1 minute, 30 times. The PCR product was mixed with pCR II-TOPO vector attached to TOPO TA cloning kit (Invitrogen) and the mixture was allowed to stand at room temperature for 5 minutes. Then, according to a conventional manner, *E. coli* Top 10 attached to the kit was transformed and applied to a LB (Amp^+) plate (containing 100 µg/ml of ampicillin). According to a conventional manner, a plasmid was extracted from each colony obtained and its nucleotide sequence was determined by cycle sequencing method with a fluorescence sequencer (ABI). Homology of the sequence of each clone was examined by means of GenBank. Regarding an unknown sequence, i.e., BSSP2 gene, the full length cDNA was obtained by 5' RACE and 3' RACE and, according to the same manner as described above, the nucleotide sequence was determined. Namely, BSSP2 clone specific primers,

GSP1 primers [mBSSP2.2 (SEQ ID NO: 27) or mBSSP2.0 (SEQ ID NO: 22)] and GSP2 primers [mBSSP2R2 (SEQ ID NO: 28) or mBSSP2.1 (SEQ ID NO: 23)] were prepared. PCR was carried out by using mouse brain Marathon-Ready cDNA (Clontech), AP1 primer attached to this reagent and either of the above GSP1 primers and heating at 94°C for 2 minutes once and repeating a cycle of heating at 94°C for 30 seconds, at 60°C for 30 seconds and then at 72°C for 30 seconds 35 times. Then, 5 µl of the PCR product diluted to 1/100, 5 µl of 10 x buffer, 5 µl of dNTP, 10 pmol of either of 10 µM of the above GSP2 primer, 10 pmol of AP2 primer attached to the above reagent and 0.5 unit of ExTaq were admixed and adjusted to 50 µl with sterilized water. Then, according to the same manner as the above, PCR was carried out. The PCR product was cloned by the above TOPO TA cloning kit and sequenced to obtain the upstream and downstream regions of the above clone. At this time, as for a clone which seemed not to cover the full length of a protein, the specific primers shown hereinafter were prepared based on the newly found nucleotide sequence. Further, based on this sequence, the primers capable of amplifying ORF as shown hereinafter [mBSSPF7 (SEQ ID NO: 26), mBSSP2R/E (SEQ ID NO: 29)] were prepared and PCR carried out using mouse brain Marathon-ready cDNA as a template to confirm that these clones were identical. This was cloned into pCR II-TOPO vector attached to TOPO TA cloning kit to obtain the plasmid pCR II/mBSSP2 containing the full length cDNA clone. The nucleotide sequence of DNA contained in this plasmid

is shown in SEQ ID NO: 7 and the amino acid sequence of mSSP2 protein deduced from the nucleotide sequence is shown in SEQ ID NO: 8. Further, two different types of clones were obtained. The nucleotide sequences of these DNA are shown in SEQ ID NOS: 3 and 5, respectively. The amino acid sequences of mBSSP2 proteins deduced from these nucleotide sequences are shown in SEQ ID NOS: 4 and 6. These novel proteases are divided into types 1, 2 and 3. Type 1 is composed of 273 amino acids, type 2 is composed of 311 amino acids and type 3 is composed of 445 amino acids. These amino acid sequences contained the common amino acid sequence composed of 238 amino acids whose N-terminus side started with Ile-Val-Gly-Gly-Gln-Ala-Val (amino acid 1-7 of SEQ ID NO:2) as the mature serine protease. Further, in the amino acid sequence of the mature serine protease, a consensus sequence having a serine protease activity was contained. Furthermore, since there were two or more amino acid sequences specific for a sugar chain bonding site, it was presumed that the amino acid sequence had at least two sugar chains.

The paragraph beginning at the bottom of page 56, line 24, has been amended as follows:

Amplification was carried out by using the primers having the sequences represented by SEQ ID NOS: 15 and 16 so that the peptide of Leu-Val-His-Gly (SEQ ID NO:43) was present at the

C-terminus of the part from trypsin signal to the enterokinase recognition site of pSecTrypHis/neurosin. This was inserted between NheI and HindIII sites of pSecTag2A to construct the plasmid pTrypSig.

The paragraph beginning at page 61, line 3, has been amended as follows:

Reverse transcription of 1 µg of mRNA of human fetus brain (Clontech) was carried out by using Superscript II (Gibco BRL) and oligo dT-Not I primer (5' GGCCACGCGTCGACTAGTA $C(T)_{17}$ 3' SEQ ID NO:44) to obtain cDNA. By using this as a template, PCR was carried out with primes prepared from mBSSP2 nucleotide sequence and represented by SEQ ID NOS: 30 and 31 to obtain a cDNA fragment of hBSSP2. Namely, 5 µl of the template, 5 µl of 10 x ExTaq buffer (TAKARA), 5 µl of dNTPs, 10 pmol portions of the above primers and 0.5 µl of ExTaq (TAKARA) were adjusted to 50 µl with sterilized water and PCR was carried out by repeating a cycle of heating at 94°C for 0.5 minute, at 55°C for 0.5 minute and then at 72°C for 1 minute, 35 times. The PCR reactions described hereinafter were carried out according to the same manner as the above composition and conditions except the template and primers. The PCR product was mixed with pGEM-T Easy vector (Promega) and Takara Ligation Solution I (TAKARA) and the reaction was carried out at 16°C for 2 hours. Then, according to the same manner, *E. coli* JM109 was transformed and applied to a LB (Amp^+) plate. A

plasmid was extracted from each colony formed according to a conventional manner and its nucleotide sequence was determined by dideoxy method. As for a clone having homology to mBSSP2, full length cDNA was obtained by 5' RACE and 3' RACE and its sequence was determined as described above. PCR was carried out by using the above cDNA as a template and primers having the sequences represented by SEQ ID NOS: 30 and 37. 3' RACE was carried out by PCR using a 1/100 dilution of the above PCR product as a template and primers having the sequences represented by SEQ ID NOS: 32 and 37. As for 5' RACE, cDNA for RACE was prepared from human fetal brain mRNA (Clontech) by using Superscript II and SMART RACE cDNA amplification kit (Clontech). PCR of this cDNA was carried out by using a primer of 10 x Universal Primer Mix (attached to the kit) and a primer having the sequence represented by SEQ ID NO: 33. Further, PCR was carried out by using the 1/100 dilution of the latter PCR product, a template, Nested PCR Primer (attached to the kit) and a primer having the sequence represented by SEQ ID NO: 34. The finally obtained PCR product was subjected to TA cloning as described above and the nucleotide sequence was determined to obtain the upstream and downstream regions of the above clone. In addition, primers for amplifying the full length cDNA as represented by SEQ ID NOS: 35 and 36 were prepared based on the resultant nucleotide sequence and PCR was carried out by using the above synthetic cDNA as a template. This PCR product was cloned into pGEM-T Easy vector to obtain the plasmid pGEM-TE/hBSSP2

containing the full length cDNA clone. The DNA sequence contained in this plasmid is shown in SEQ ID NO: 9 and hBSSP2 protein deduced from the nucleotide sequence is shown in SEQ ID NO: 10.

SEQUENCE LISTING

<110> UEMURA, Hidetoshi
OKUI, Akira
KOMINAMI, Katsuya
YAMAGUCHI, Nozomi
MITSUI, Shinichi

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Pro His Trp Val Val Thr Ala Ala His Cys Met Tyr Ser Phe Arg Leu
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1 5 10

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15                  20                  25                  30

cct ctg gct tct cga ata gtt ggc ggc caa gct gtg gct tct ggg cgc      384
Pro Leu Ala Ser Arg Ile Val Gly Gly Gln Ala Val Ala Ser Gly Arg
                35                  40                  45

tgg cca tgg caa gct agc gtg atg ctt ggc tcc cgg cac acg tgt ggg      432
Trp Pro Trp Gln Ala Ser Val Met Leu Gly Ser Arg His Thr Cys Gly
            50                  55                  60

gcc tct gtg ttg gca cca cac tgg gta gtg act gct gcc cac tgc atg      480
Ala Ser Val Leu Ala Pro His Trp Val Val Thr Ala Ala His Cys Met
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tac agt ttc agg ctg tcc cgc cta tcc agc tgg cgg gtt cat gca ggg      528
Tyr Ser Phe Arg Leu Ser Arg Leu Ser Ser Trp Arg Val His Ala Gly
    80                  85                  90

ctg gtc agc cat ggt gct gtc cga caa cac cag gga act atg gtg gag      576
Leu Val Ser His Gly Ala Val Arg Gln His Gln Gly Thr Met Val Glu
95                  100                 105                 110

aag atc att cct cat cct ttg tac agt gcc cag aac cat gac tat gat      624
Lys Ile Ile Pro His Pro Leu Tyr Ser Ala Gln Asn His Asp Tyr Asp
                115                 120                 125

gtg gct ctg ctg cag ctc cgg aca cca atc aac ttc tca gac acc gtg      672
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255                 260                 265                 270
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cag gtc cgc tagccgaaga agcagcagca gccacctgtg acgccgagct 1105
Gln Val Arg

gtggatcgcc catggatcac cccagtctgg gggccagcat ctgggtcact gggcctctcc 1165

ccaaaggctc tgacttcgag ttcatctttc tcatctgaga acctccacaa caggaaaagg 1225

agtctgcggc tagattggga atgatggtga gaggaaggga taggaggaca gaagagacag 1285

cagaggcttc tggaagcatc tgggagactg ctcctctgct ccccccacac cccacgtgca 1345

tccactgggg gatgctggag atgcccaatc cttgtttctt gtggggccac tggaaggcta 1405

agtccaactt tagaggatgc cctgtctcga gagttactag gcagataagg ttaaggttgg 1465

acaagctcag gtaaaggcac ggaagtcaag atcccctctc ccccgtgcgg tcctgttctg 1525

aggtaagcta atagccccgc accaggcaga ggtctacagg gtaagaagga tgcagttggg 1585

ctacacgacg ctatttttca aatgatgttt ctgtaaattg gttgagagag ttttgttatt 1645

aaacagaaat tatgtataaa aaaaaaaaaa aaaaaaaaaa 1685

<210> 4
<211> 273
<212> PRT
<213> Mus sp.

<400> 4

Met Glu Ala Gln Val Gly Leu Leu Trp Val Ser Ala Asn Cys Pro Ser
1 5 10 15

Gly Arg Ile Val Ser Leu Lys Cys Ser Glu Cys Gly Ala Arg Pro Leu
20 25 30

Ala Ser Arg Ile Val Gly Gly Gln Ala Val Ala Ser Gly Arg Trp Pro
35 40 45

Trp Gln Ala Ser Val Met Leu Gly Ser Arg His Thr Cys Gly Ala Ser
50 55 60

Val Leu Ala Pro His Trp Val Val Thr Ala Ala His Cys Met Tyr Ser
65 70 75 80

Phe Arg Leu Ser Arg Leu Ser Ser Trp Arg Val His Ala Gly Leu Val
85 90 95

Ser His Gly Ala Val Arg Gln His Gln Gly Thr Met Val Glu Lys Ile
100 105 110

Ile Pro His Pro Leu Tyr Ser Ala Gln Asn His Asp Tyr Asp Val Ala
115 120 125

Leu Leu Gln Leu Arg Thr Pro Ile Asn Phe Ser Asp Thr Val Asp Ala
130 135 140

Val Cys Leu Pro Ala Lys Glu Gln Tyr Phe Pro Trp Gly Ser Gln Cys
145 150 155 160

Trp Val Ser Gly Trp Gly His Thr Asp Pro Ser His Thr His Ser Ser
165 170 175

Asp Thr Leu Gln Asp Thr Met Val Pro Leu Leu Ser Thr His Leu Cys
180 185 190

Asn Ser Ser Cys Met Tyr Ser Gly Ala Leu Thr His Arg Met Leu Cys
195 200 205

Ala Gly Tyr Leu Asp Gly Arg Ala Asp Ala Cys Gln Gly Asp Ser Gly
210 215 220

Gly Pro Leu Val Cys Pro Ser Gly Asp Thr Trp His Leu Val Gly Val
225 230 235 240

Val Ser Trp Gly Arg Gly Cys Ala Glu Pro Asn Arg Pro Gly Val Tyr
245 250 255

Ala Lys Val Ala Glu Phe Leu Asp Trp Ile His Asp Thr Val Gln Val
260 265 270

Arg

<210> 5
<211> 2068
<212> DNA
<213> Mus sp.

<220>
<221> CDS
<222> (516)..(1448)
<223>

<400> 5
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acttactgcc ttatatcagt gcagctgact catatgagtc caacactgga tgaccaaagc 120

ccaatggaga ttcggtgcac ggaagagggt gctgggcctg ggatcttcag aatggagttg 180

ggagaccaga ggcaatccat ttctcagtcc caacgctggt gctgcctgca acgtggctgt 240

gtaatactgg gcgtcctggg gctgctggct ggagcaggca ttgcttcatg gctcttagtg 300

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ttgtatctat ggccggctgc ctctccatcc atctctggga cgttgcagga ggaggagatg     360

actttgaact gtccaggagt gagctgtgag gaagagctcc ttccatctct tcccaaaaca     420

gaataaatgg aggggatctt ctgcttcaag tacaagtaag agctcggcca gactggctcc     480

tggtctgcca tgagggctgg agccccgccc tgggc atg cac atc tgc aag agt        533
                                        Met His Ile Cys Lys Ser
                                        1               5

ctt ggg cat atc agg ctt act caa cac aag gcc gtg aat ctg tct gac       581
Leu Gly His Ile Arg Leu Thr Gln His Lys Ala Val Asn Leu Ser Asp
            10                  15                  20

atc aag ctc aac aga tcc cag gag ttt gct caa ctc tct gct aga ccg       629
Ile Lys Leu Asn Arg Ser Gln Glu Phe Ala Gln Leu Ser Ala Arg Pro
        25                  30                  35

gga ggc ctt gta gag gag gca tgg aag ccc agc gct aac tgt cct tct       677
Gly Gly Leu Val Glu Glu Ala Trp Lys Pro Ser Ala Asn Cys Pro Ser
    40                  45                  50

ggc cga att gtt tct ctc aaa tgt tct gag tgt ggg gca agg cct ctg       725
Gly Arg Ile Val Ser Leu Lys Cys Ser Glu Cys Gly Ala Arg Pro Leu
55                  60                  65                  70

gct tct cga ata gtt ggc ggc caa gct gtg gct tct ggg cgc tgg cca       773
Ala Ser Arg Ile Val Gly Gly Gln Ala Val Ala Ser Gly Arg Trp Pro
                75                  80                  85

tgg caa gct agc gtg atg ctt ggc tcc cgg cac acg tgt ggg gcc tct       821
Trp Gln Ala Ser Val Met Leu Gly Ser Arg His Thr Cys Gly Ala Ser
            90                  95                  100

gtg ttg gca cca cac tgg gta gtg act gct gcc cac tgc atg tac agt       869
Val Leu Ala Pro His Trp Val Val Thr Ala Ala His Cys Met Tyr Ser
        105                 110                 115

ttc agg ctg tcc cgc cta tcc agc tgg cgg gtt cat gca ggg ctg gtc       917
Phe Arg Leu Ser Arg Leu Ser Ser Trp Arg Val His Ala Gly Leu Val
    120                 125                 130

agc cat ggt gct gtc cga caa cac cag gga act atg gtg gag aag atc       965
Ser His Gly Ala Val Arg Gln His Gln Gly Thr Met Val Glu Lys Ile
135                 140                 145                 150

att cct cat cct ttg tac agt gcc cag aac cat gac tat gat gtg gct      1013
Ile Pro His Pro Leu Tyr Ser Ala Gln Asn His Asp Tyr Asp Val Ala
                155                 160                 165

ctg ctg cag ctc cgg aca cca atc aac ttc tca gac acc gtg gac gct      1061
Leu Leu Gln Leu Arg Thr Pro Ile Asn Phe Ser Asp Thr Val Asp Ala
            170                 175                 180

gtg tgc ttg ccg gcc aag gag cag tac ttt cca tgg ggg tcg cag tgc      1109
Val Cys Leu Pro Ala Lys Glu Gln Tyr Phe Pro Trp Gly Ser Gln Cys
        185                 190                 195

tgg gtg tct ggc tgg ggc cac acc gac ccc agc cat act cat agc tca      1157
Trp Val Ser Gly Trp Gly His Thr Asp Pro Ser His Thr His Ser Ser
    200                 205                 210
```

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gat aca ctg cag gac aca atg gta ccc ctg ctc agc acc cac ctc tgc     1205
Asp Thr Leu Gln Asp Thr Met Val Pro Leu Leu Ser Thr His Leu Cys
215                 220                 225                 230

aac agc tca tgc atg tac agt ggg gca ctt aca cac cgc atg ttg tgt     1253
Asn Ser Ser Cys Met Tyr Ser Gly Ala Leu Thr His Arg Met Leu Cys
                235                 240                 245

gct ggc tac ctg gat gga agg gca gac gca tgc cag gga gac agc ggg     1301
Ala Gly Tyr Leu Asp Gly Arg Ala Asp Ala Cys Gln Gly Asp Ser Gly
            250                 255                 260

gga ccc ctg gta tgt ccc agt ggt gac acg tgg cac ctt gta ggg gtg     1349
Gly Pro Leu Val Cys Pro Ser Gly Asp Thr Trp His Leu Val Gly Val
        265                 270                 275

gtc agc tgg ggt cgt ggc tgt gca gag ccc aat cgc cca ggt gtc tat     1397
Val Ser Trp Gly Arg Gly Cys Ala Glu Pro Asn Arg Pro Gly Val Tyr
    280                 285                 290

gcc aag gta gca gag ttc ctg gac tgg atc cat gac act gtg cag gtc     1445
Ala Lys Val Ala Glu Phe Leu Asp Trp Ile His Asp Thr Val Gln Val
295                 300                 305                 310

cgc tagccgaaga agcagcagca gccacctgtg acgccgagct gtggatcgcc          1498
Arg
```

```
catggatcac cccagtctgg gggccagcat ctgggtcact gggcctctcc ccaaaggctc   1558

tgacttcgag ttcatctttc tcatctgaga acctccacaa caggaaaagg agtctgcggc   1618

tagattggga atgatggtga gaggaaggga taggaggaca gaagagacag cagaggcttc   1678

tggaagcatc tgggagactg ctcctctgct ccccccacac cccacgtgca tccactgggg   1738

gatgctggag atgcccaatc cttgtttctt gtggggccac tggaaggcta agtccaactt   1798

tagaggatgc cctgtctcga gagttactag gcagataagg ttaaggttgg acaagctcag   1858

gtaaaggcac ggaagtcaag atcccctctc ccccgtgcgg tcctgttctg aggtaagcta   1918

atagccccgc accaggcaga ggtctacagg gtaagaagga tgcagttggg ctacacgacg   1978

ctatttttca aatgatgttt ctgtaaattg gttgagagag ttttgttatt aaacagaaat   2038

tatgtataaa aaaaaaaaaa aaaaaaaaaa                                    2068
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<210> 6
<211> 311
<212> PRT
<213> Mus sp.

<400> 6

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Met His Ile Cys Lys Ser Leu Gly His Ile Arg Leu Thr Gln His Lys
1               5                   10                  15

Ala Val Asn Leu Ser Asp Ile Lys Leu Asn Arg Ser Gln Glu Phe Ala
            20                  25                  30
```

Gln Leu Ser Ala Arg Pro Gly Gly Leu Val Glu Glu Ala Trp Lys Pro
35 40 45

Ser Ala Asn Cys Pro Ser Gly Arg Ile Val Ser Leu Lys Cys Ser Glu
50 55 60

Cys Gly Ala Arg Pro Leu Ala Ser Arg Ile Val Gly Gly Gln Ala Val
65 70 75 80

Ala Ser Gly Arg Trp Pro Trp Gln Ala Ser Val Met Leu Gly Ser Arg
85 90 95

His Thr Cys Gly Ala Ser Val Leu Ala Pro His Trp Val Val Thr Ala
100 105 110

Ala His Cys Met Tyr Ser Phe Arg Leu Ser Arg Leu Ser Ser Trp Arg
115 120 125

Val His Ala Gly Leu Val Ser His Gly Ala Val Arg Gln His Gln Gly
130 135 140

Thr Met Val Glu Lys Ile Ile Pro His Pro Leu Tyr Ser Ala Gln Asn
145 150 155 160

His Asp Tyr Asp Val Ala Leu Leu Gln Leu Arg Thr Pro Ile Asn Phe
165 170 175

Ser Asp Thr Val Asp Ala Val Cys Leu Pro Ala Lys Glu Gln Tyr Phe
180 185 190

Pro Trp Gly Ser Gln Cys Trp Val Ser Gly Trp Gly His Thr Asp Pro
195 200 205

Ser His Thr His Ser Ser Asp Thr Leu Gln Asp Thr Met Val Pro Leu
210 215 220

Leu Ser Thr His Leu Cys Asn Ser Ser Cys Met Tyr Ser Gly Ala Leu
225 230 235 240

Thr His Arg Met Leu Cys Ala Gly Tyr Leu Asp Gly Arg Ala Asp Ala
245 250 255

Cys Gln Gly Asp Ser Gly Gly Pro Leu Val Cys Pro Ser Gly Asp Thr
260 265 270

Trp His Leu Val Gly Val Val Ser Trp Gly Arg Gly Cys Ala Glu Pro
275 280 285

```
Asn Arg Pro Gly Val Tyr Ala Lys Val Ala Glu Phe Leu Asp Trp Ile
    290                 295                 300

His Asp Thr Val Gln Val Arg
305                 310


<210>  7
<211>  2070
<212>  DNA
<213>  Mus sp.

<220>
<221>  CDS
<222>  (116)..(1450)
<223>


<400>  7
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gctggatctt caaccactat ttctccagag tccaacactg gatgaccaaa gccca atg      118
                                                              Met
                                                               1

gag att cgg tgc acg gaa gag ggt gct ggg cct ggg atc ttc aga atg      166
Glu Ile Arg Cys Thr Glu Glu Gly Ala Gly Pro Gly Ile Phe Arg Met
            5                   10                  15

gag ttg gga gac cag agg caa tcc att tct cag tcc caa cgc tgg tgc      214
Glu Leu Gly Asp Gln Arg Gln Ser Ile Ser Gln Ser Gln Arg Trp Cys
        20                  25                  30

tgc ctg caa cgt ggc tgt gta ata ctg ggc gtc ctg ggg ctg ctg gct      262
Cys Leu Gln Arg Gly Cys Val Ile Leu Gly Val Leu Gly Leu Leu Ala
    35                  40                  45

gga gca ggc att gct tca tgg ctc tta gtg ttg tat cta tgg cca gct      310
Gly Ala Gly Ile Ala Ser Trp Leu Leu Val Leu Tyr Leu Trp Pro Ala
50                  55                  60                  65

gcc tct cca tcc atc tct ggg acg ttg cag gag gag gag atg act ttg      358
Ala Ser Pro Ser Ile Ser Gly Thr Leu Gln Glu Glu Glu Met Thr Leu
                70                  75                  80

aac tgt cca gga gtg agc tgt gag gaa gag ctc ctt cca tct ctt ccc      406
Asn Cys Pro Gly Val Ser Cys Glu Glu Glu Leu Leu Pro Ser Leu Pro
            85                  90                  95

aaa aca gta tct ttc aga ata aat gga gag gat ctt ctg ctt caa gta      454
Lys Thr Val Ser Phe Arg Ile Asn Gly Glu Asp Leu Leu Leu Gln Val
        100                 105                 110

caa gta aga gct cgg cca gac tgg ctc ctg gtc tgc cat gag ggc tgg      502
Gln Val Arg Ala Arg Pro Asp Trp Leu Leu Val Cys His Glu Gly Trp
    115                 120                 125

agc ccc gcc ctg ggc atg cac atc tgc aag agt ctt ggg cat atc agg      550
Ser Pro Ala Leu Gly Met His Ile Cys Lys Ser Leu Gly His Ile Arg
130                 135                 140                 145
```

```
ctt act caa cac aag gcc gtg aat ctg tct gac atc aag ctc aac aga      598
Leu Thr Gln His Lys Ala Val Asn Leu Ser Asp Ile Lys Leu Asn Arg
                150                 155                 160

tcc cag gag ttt gct caa ctc tct gct aga ccg gga ggc ctt gta gag      646
Ser Gln Glu Phe Ala Gln Leu Ser Ala Arg Pro Gly Gly Leu Val Glu
            165                 170                 175

gag gca tgg aag ccc agc gct aac tgt cct tct ggc cga att gtt tct      694
Glu Ala Trp Lys Pro Ser Ala Asn Cys Pro Ser Gly Arg Ile Val Ser
        180                 185                 190

ctc aaa tgt tct gag tgt ggg gca agg cct ctg gct tct cga ata gtt      742
Leu Lys Cys Ser Glu Cys Gly Ala Arg Pro Leu Ala Ser Arg Ile Val
    195                 200                 205

ggc ggc caa gct gtg gct tct ggg cgc tgg cca tgg caa gct agc gtg      790
Gly Gly Gln Ala Val Ala Ser Gly Arg Trp Pro Trp Gln Ala Ser Val
210                 215                 220                 225

atg ctt ggc tcc cgg cac acg tgt ggg gcc tct gtg ttg gca cca cac      838
Met Leu Gly Ser Arg His Thr Cys Gly Ala Ser Val Leu Ala Pro His
                230                 235                 240

tgg gta gtg act gct gcc cac tgc atg tac agt ttc agg ctg tcc cgc      886
Trp Val Val Thr Ala Ala His Cys Met Tyr Ser Phe Arg Leu Ser Arg
            245                 250                 255

cta tcc agc tgg cgg gtt cat gca ggg ctg gtc agc cat ggt gct gtc      934
Leu Ser Ser Trp Arg Val His Ala Gly Leu Val Ser His Gly Ala Val
        260                 265                 270

cga caa cac cag gga act atg gtg gag aag atc att cct cat cct ttg      982
Arg Gln His Gln Gly Thr Met Val Glu Lys Ile Ile Pro His Pro Leu
    275                 280                 285

tac agt gcc cag aac cat gac tat gat gtg gct ctg ctg cag ctc cgg     1030
Tyr Ser Ala Gln Asn His Asp Tyr Asp Val Ala Leu Leu Gln Leu Arg
290                 295                 300                 305

aca cca atc aac ttc tca gac acc gtg gac gct gtg tgc ttg ccg gcc     1078
Thr Pro Ile Asn Phe Ser Asp Thr Val Asp Ala Val Cys Leu Pro Ala
                310                 315                 320

aag gag cag tac ttt cca tgg ggg tcg cag tgc tgg gtg tct ggc tgg     1126
Lys Glu Gln Tyr Phe Pro Trp Gly Ser Gln Cys Trp Val Ser Gly Trp
            325                 330                 335

ggc cac acc gac ccc agc cat act cat agc tca gat aca ctg cag gac     1174
Gly His Thr Asp Pro Ser His Thr His Ser Ser Asp Thr Leu Gln Asp
        340                 345                 350

aca atg gta ccc ctg ctc agc acc cac ctc tgc aac agc tca tgc atg     1222
Thr Met Val Pro Leu Leu Ser Thr His Leu Cys Asn Ser Ser Cys Met
    355                 360                 365

tac agt ggg gca ctt aca cac cgc atg ttg tgt gct ggc tac ctg gat     1270
Tyr Ser Gly Ala Leu Thr His Arg Met Leu Cys Ala Gly Tyr Leu Asp
370                 375                 380                 385

gga agg gca gac gca tgc cag gga gac agc ggg gga ccc ctg gta tgt     1318
Gly Arg Ala Asp Ala Cys Gln Gly Asp Ser Gly Gly Pro Leu Val Cys
```

```
                390                 395                 400

ccc agt ggt gac acg tgg cac ctt gta ggg gtg gtc agc tgg ggt cgt      1366
Pro Ser Gly Asp Thr Trp His Leu Val Gly Val Val Ser Trp Gly Arg
            405                 410                 415

ggc tgt gca gag ccc aat cgc cca ggt gtc tat gcc aag gta gca gag      1414
Gly Cys Ala Glu Pro Asn Arg Pro Gly Val Tyr Ala Lys Val Ala Glu
        420                 425                 430

ttc ctg gac tgg atc cat gac act gtg cag gtc cgc tagccgaaga           1460
Phe Leu Asp Trp Ile His Asp Thr Val Gln Val Arg
    435                 440                 445

agcagcagca gccacctgtg acgccgagct gtggatcgcc catggatcac cccagtctgg    1520

gggccagcat ctgggtcact gggcctctcc ccaaaggctc tgacttcgag ttcatctttc    1580

tcatctgaga acctccacaa caggaaaagg agtctgcggc tagattggga atgatggtga    1640

gaggaaggga taggaggaca gaagagacag cagaggcttc tggaagcatc tgggagactg    1700

ctcctctgct ccccccacac cccacgtgca tccactgggg gatgctggag atgcccaatc    1760

cttgtttctt gtggggccac tggaaggcta agtccaactt tagaggatgc cctgtctcga    1820

gagttactag gcagataagg ttaaggttgg acaagctcag gtaaaggcac ggaagtcaag    1880

atcccctctc ccccgtgcgg tcctgttctg aggtaagcta atagccccgc accaggcaga    1940

ggtctacagg gtaagaagga tgcagttggg ctacacgacg ctatttttca aatgatgttt    2000

ctgtaaattg gttgagagag ttttgttatt aaacagaaat tatgtataaa aaaaaaaaaa    2060

aaaaaaaaaa                                                           2070
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<210> 8
<211> 445
<212> PRT
<213> Mus sp.

<400> 8

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Met Glu Ile Arg Cys Thr Glu Glu Gly Ala Gly Pro Gly Ile Phe Arg
1               5                  10                  15

Met Glu Leu Gly Asp Gln Arg Gln Ser Ile Ser Gln Ser Gln Arg Trp
            20                  25                  30

Cys Cys Leu Gln Arg Gly Cys Val Ile Leu Gly Val Leu Gly Leu Leu
        35                  40                  45

Ala Gly Ala Gly Ile Ala Ser Trp Leu Leu Val Leu Tyr Leu Trp Pro
    50                  55                  60

Ala Ala Ser Pro Ser Ile Ser Gly Thr Leu Gln Glu Glu Glu Met Thr
65                  70                  75                  80
```

Leu Asn Cys Pro Gly Val Ser Cys Glu Glu Glu Leu Leu Pro Ser Leu
85 90 95

Pro Lys Thr Val Ser Phe Arg Ile Asn Gly Glu Asp Leu Leu Leu Gln
100 105 110

Val Gln Val Arg Ala Arg Pro Asp Trp Leu Leu Val Cys His Glu Gly
115 120 125

Trp Ser Pro Ala Leu Gly Met His Ile Cys Lys Ser Leu Gly His Ile
130 135 140

Arg Leu Thr Gln His Lys Ala Val Asn Leu Ser Asp Ile Lys Leu Asn
145 150 155 160

Arg Ser Gln Glu Phe Ala Gln Leu Ser Ala Arg Pro Gly Gly Leu Val
165 170 175

Glu Glu Ala Trp Lys Pro Ser Ala Asn Cys Pro Ser Gly Arg Ile Val
180 185 190

Ser Leu Lys Cys Ser Glu Cys Gly Ala Arg Pro Leu Ala Ser Arg Ile
195 200 205

Val Gly Gly Gln Ala Val Ala Ser Gly Arg Trp Pro Trp Gln Ala Ser
210 215 220

Val Met Leu Gly Ser Arg His Thr Cys Gly Ala Ser Val Leu Ala Pro
225 230 235 240

His Trp Val Val Thr Ala Ala His Cys Met Tyr Ser Phe Arg Leu Ser
245 250 255

Arg Leu Ser Ser Trp Arg Val His Ala Gly Leu Val Ser His Gly Ala
260 265 270

Val Arg Gln His Gln Gly Thr Met Val Glu Lys Ile Ile Pro His Pro
275 280 285

Leu Tyr Ser Ala Gln Asn His Asp Tyr Asp Val Ala Leu Leu Gln Leu
290 295 300

Arg Thr Pro Ile Asn Phe Ser Asp Thr Val Asp Ala Val Cys Leu Pro
305 310 315 320

Ala Lys Glu Gln Tyr Phe Pro Trp Gly Ser Gln Cys Trp Val Ser Gly
325 330 335

Trp Gly His Thr Asp Pro Ser His Thr His Ser Ser Asp Thr Leu Gln
340 345 350

Asp Thr Met Val Pro Leu Leu Ser Thr His Leu Cys Asn Ser Ser Cys
355 360 365

Met Tyr Ser Gly Ala Leu Thr His Arg Met Leu Cys Ala Gly Tyr Leu
370 375 380

Asp Gly Arg Ala Asp Ala Cys Gln Gly Asp Ser Gly Gly Pro Leu Val
385 390 395 400

Cys Pro Ser Gly Asp Thr Trp His Leu Val Gly Val Val Ser Trp Gly
405 410 415

Arg Gly Cys Ala Glu Pro Asn Arg Pro Gly Val Tyr Ala Lys Val Ala
420 425 430

Glu Phe Leu Asp Trp Ile His Asp Thr Val Gln Val Arg
435 440 445

<210> 9
<211> 2265
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (156)..(1526)
<223>

<400> 9
acgcgggata cagggagggg ccatgtgcga accagggaga cctcatcttc caaccaagct 60

tgctgggctt gcatttaatc aatgcatggc cagagaacag gagcggaaca ttgcctagta 120

gaccctgagg ctttacaaca gtgctactga ccccct atg agc ctg atg ctg gat 173
Met Ser Leu Met Leu Asp
1 5

gac caa ccc cct atg gag gcc cag tat gca gag gag ggc cca gga cct 221
Asp Gln Pro Pro Met Glu Ala Gln Tyr Ala Glu Glu Gly Pro Gly Pro
10 15 20

ggg atc ttc aga gca gag cct gga gac cag cag cat ccc att tct cag 269
Gly Ile Phe Arg Ala Glu Pro Gly Asp Gln Gln His Pro Ile Ser Gln
25 30 35

gcg gtg tgc tgg cgt tcc atg cga cgt ggc tgt gca gtg ctg gga gcc 317
Ala Val Cys Trp Arg Ser Met Arg Arg Gly Cys Ala Val Leu Gly Ala
40 45 50

ctg ggg ctg ctg gcc ggt gca ggt gtt ggc tca tgg ctc cta gtg ctg 365

```
Leu Gly Leu Leu Ala Gly Ala Gly Val Gly Ser Trp Leu Leu Val Leu
55                  60                  65                  70

tat ctg tgt cct gct gcc tct cag ccc att tcc ggg acc ttg cag gat      413
Tyr Leu Cys Pro Ala Ala Ser Gln Pro Ile Ser Gly Thr Leu Gln Asp
                75                  80                  85

gag gag ata act ttg agc tgc tca gag gcc agc gct gag gaa gct ctg      461
Glu Glu Ile Thr Leu Ser Cys Ser Glu Ala Ser Ala Glu Glu Ala Leu
            90                  95                  100

ctc cct gca ctc ccc aaa aca gta tct ttc aga ata aac agc gaa gac      509
Leu Pro Ala Leu Pro Lys Thr Val Ser Phe Arg Ile Asn Ser Glu Asp
        105                 110                 115

ttc ttg ctg gaa gcg caa gtg agg gat cag cca cgc tgg ctc ctg gtc      557
Phe Leu Leu Glu Ala Gln Val Arg Asp Gln Pro Arg Trp Leu Leu Val
    120                 125                 130

tgc cat gag ggc tgg agc ccc gcc ctg ggg ctg cag atc tgc tgg agc      605
Cys His Glu Gly Trp Ser Pro Ala Leu Gly Leu Gln Ile Cys Trp Ser
135                 140                 145                 150

ctt ggg cat ctc aga ctc act cac cac aag gga gta aac ctc act gac      653
Leu Gly His Leu Arg Leu Thr His His Lys Gly Val Asn Leu Thr Asp
                155                 160                 165

atc aaa ctc aac agt tcc cag gag ttt gct cag ctc tct cct aga ctg      701
Ile Lys Leu Asn Ser Ser Gln Glu Phe Ala Gln Leu Ser Pro Arg Leu
            170                 175                 180

gga ggc ttc ctg gag gag gcg tgg cag ccc agg aac aac tgc act tct      749
Gly Gly Phe Leu Glu Glu Ala Trp Gln Pro Arg Asn Asn Cys Thr Ser
        185                 190                 195

ggt caa gtt gtt tcc ctc aga tgc tct gag tgt gga gcg agg ccc ctg      797
Gly Gln Val Val Ser Leu Arg Cys Ser Glu Cys Gly Ala Arg Pro Leu
    200                 205                 210

gct tcc cgg ata gtt ggt ggg cag tct gtg gct cct ggg cgc tgg ccg      845
Ala Ser Arg Ile Val Gly Gly Gln Ser Val Ala Pro Gly Arg Trp Pro
215                 220                 225                 230

tgg cag gcc agc gtg gcc ctg ggc ttc cgg cac acg tgt ggg ggc tct      893
Trp Gln Ala Ser Val Ala Leu Gly Phe Arg His Thr Cys Gly Gly Ser
                235                 240                 245

gtg cta gcg cca cgc tgg gtg gtg act gct gca cat tgt atg cac agt      941
Val Leu Ala Pro Arg Trp Val Val Thr Ala Ala His Cys Met His Ser
            250                 255                 260

ttc agg ctg gcc cgc ctg tcc agc tgg cgg gtt cat gcg ggg ctg gtc      989
Phe Arg Leu Ala Arg Leu Ser Ser Trp Arg Val His Ala Gly Leu Val
        265                 270                 275

agc cac agt gcc gtc agg ccc cac caa ggg gct ctg gtg gag agg att     1037
Ser His Ser Ala Val Arg Pro His Gln Gly Ala Leu Val Glu Arg Ile
    280                 285                 290

atc cca cac ccc ctc tac agt gcc cag aat cat gac tac gac gtc gcc     1085
Ile Pro His Pro Leu Tyr Ser Ala Gln Asn His Asp Tyr Asp Val Ala
295                 300                 305                 310
```

```
ctc ctg agg ctc cag acc gct ctc aac ttc tca gac act gtg ggc gct     1133
Leu Leu Arg Leu Gln Thr Ala Leu Asn Phe Ser Asp Thr Val Gly Ala
                315                 320                 325

gtg tgc ctg ccg gcc aag gaa cag cat ttt ccg aag ggc tcg cgg tgc     1181
Val Cys Leu Pro Ala Lys Glu Gln His Phe Pro Lys Gly Ser Arg Cys
            330                 335                 340

tgg gtg tct ggc tgg ggc cac acc cac cct agc cat act tac agc tcg     1229
Trp Val Ser Gly Trp Gly His Thr His Pro Ser His Thr Tyr Ser Ser
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gat atg ctc cag gac acg gtg gtg ccc ttg ttc agc act cag ctc tgc     1277
Asp Met Leu Gln Asp Thr Val Val Pro Leu Phe Ser Thr Gln Leu Cys
    360                 365                 370

aac agc tct tgc gtg tac agc gga gcc ctc acc ccc cgc atg ctt tgc     1325
Asn Ser Ser Cys Val Tyr Ser Gly Ala Leu Thr Pro Arg Met Leu Cys
375                 380                 385                 390

gct ggc tac ctg gac gga agg gct gat gca tgc cag gga gat agc ggg     1373
Ala Gly Tyr Leu Asp Gly Arg Ala Asp Ala Cys Gln Gly Asp Ser Gly
                395                 400                 405

ggc ccc cta gtg tgc cca gat ggg gac aca tgg cgc cta gtg ggg gtg     1421
Gly Pro Leu Val Cys Pro Asp Gly Asp Thr Trp Arg Leu Val Gly Val
            410                 415                 420

gtc agc tgg ggg cgt gcg tgc gca gag ccc aat cac cca ggt gtc tac     1469
Val Ser Trp Gly Arg Ala Cys Ala Glu Pro Asn His Pro Gly Val Tyr
        425                 430                 435

gcc aag gta gct gag ttt ctg gac tgg atc cat gac act gct cag gac     1517
Ala Lys Val Ala Glu Phe Leu Asp Trp Ile His Asp Thr Ala Gln Asp
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tcc ctc ctc tgagtcctgc tgtttcctcc agtctcactg cacaccactg             1566
Ser Leu Leu
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#3

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

ATTY.'S DOCKET: UEMURA7

In re Application of:	)	Art Unit:
	)	
H. UEMURA, et al.	)	Examiner:
	)	
Serial No.: 09/856,371	)	Confirmation No.
	)	
Filed: May 21, 2001	)	Washington D.C.
	)	
For: NOVEL SERINE PROTEASE BSSP2	)	August 14, 2001
	)	

PRELIMINARY AMENDMENT

Honorable Commissioner for Patents
Washington, D.C. 20231

Prior to examination of the above-identified application, please enter the following Preliminary Amendment:

IN THE SPECIFICATION

Page 1, please amend the second paragraph as follows:

In general, proteases are biosynthesized as inactive precursors. They undergo limited hydrolysis in molecules to be converted into activated type proteases. In so far as enzymes are proteases, they have an activity for hydrolyzing a peptide bond, while their actions vary according to kinds of proteases. According to a particular kind of catalytic site, proteases are divided into serine proteases, cysteine proteases, aspartate proteases, metal proteases and the like. Proteases of each kind have a variety of properties, ranging from a protease having general digestive properties to a protease having various regulatory domains and strict substrate specificity, thereby specifically hydrolyzing

only characteristic proteins.

Page 2, please amend the paragraph on page 2 as follows:

Further, proteins undergo various types of processing even after translation to produce active proteins. In many secretory proteins, a protein is first synthesized on the ribosome in cytoplasm as an inactive precursor (pro-form) which comprises an active protein bearing at the N-terminus thereof a peptide of about 15 to 60 amino acids responsible for secretion (secretory signal). This peptide region is concerned with the mechanism for passing through the cell membrane and is removed upon cleavage by a specific protease during the passage through the membrane, in almost all the cases, to produce the mature form. A secretory signal has a broad hydrophobic region comprising hydrophobic amino acids in the middle of the sequence, and basic amino acid residues at a site close to the N-terminus. A secretory signal is a synonym for a signal peptide. In addition, in some proteins, a peptide moiety which functions as a secretory signal is further attached to the N-terminus of the inactive precursor (pro-form). Such a protein is called a prepro-protein (prepro-form).

Page 3, please amend the first paragraph as follows:

For example, trypsin is present in the form of a prepro-form immediately after translation into amino acids. After being secreted from cells, it is present in the form of a pro-form and is then converted into active trypsin in the duodenum upon limited hydrolysis by enteropeptidase or by trypsin itself.

Page 3, please amend the second paragraph as follows:

The optimal pH range of serine proteases is neutral to weak alkaline and, in general, many of them have a molecular weight of about 30,000 or lower. All proteases relating to blood coagulation, fibrinolysis and complement systems having a large molecular weight belong to the family of trypsin-like serine proteases. They have many regulator domains and form a protease cascade which is of very importance to reactions in a living body.

Page 4, please amend the first paragraph as follows:

Serine proteases expressed in a brain-nerve system such as neurosin are considered to play various roles in the brain-nerve system. Therefore, there is a possibility that isolation of a gene encoding a novel protease expressed in a brain-nerve system and production of a protein using the gene

would be useful for diagnosis or therapy of various diseases related to the brain-nerve system.

Page 4, please amend the second paragraph as follows:

Nowadays, in general, clinical diagnosis of Alzheimer's disease is based on the diagnosis standard of DSM-IIIR and NINCDS-ADRDA (Mckhann, G. et al., Neurology, 34. 939, 1994) or the diagnosis standard of DSM-IV (American Psychiatric Association; Diagnostic and statistical manuals of mental disorders, 4th ed., Washington DC, American Psychiatric Association, 1994). However, these standards are conditioned by a decline in recognition functions which causes a severe disability in daily life or social life. Then, it is pointed out that the diagnosis is less than scientifically objective because the diagnosis may be influenced by the level of an individual's social life and further the specialty and experience of a physician who diagnoses particular conditions. In addition, definite diagnosis of Alzheimer's disease is conducted by pathohistological analyses and, in this respect, substantial inconsistency between clinical diagnosis and autopsy diagnosis exists.

Page 5, please amend the paragraph on page 5 as follows:

At present, image diagnosis is employed as a supplemental means in clinical diagnosis of Alzheimer's diagnosis and it is possible to analyze brain functions, for example, decline of metabolism and atrophy in specific sites such as hippocampus, parietal lobe of cerebral cortex and the like which are specific for Alzheimer's disease by PET and SPECT. However, to define Alzheimer's disease based on lowering of a blood flow from parietal lobe to temporal lobe is very dangerous. In addition, there is a report showing that MRS test is useful for patients with dementia including those of Alzheimer's disease. Further, although CT-MRI image diagnosis is used, a lesion of white matter such as atrophy of brain, PVL or the like is not specific for Alzheimer type dementia. Since it has been reported that atrophy of brain proceeds with aging, the above observation is not necessarily found in Alzheimer type dementia. Furthermore, since an image obtained by MRI varies according to strength of a magnetic field, performance of the apparatus and imaging conditions, numerical data obtained in different facilities cannot be compared with each other except for atrophic change. In addition, there is a limit to image measurement. Further, enlargement of the ventricle can be recognized in vascular dementia cases and there are cases wherein atrophy of the

hippocampus is observed after ischemia of the basilar artery.

Page 6, please amend the fourth paragraph as follows:

Further, data obtained in different facilities can be compared with each other by using the same diagnosis marker. Therefore, development of biological diagnosis markers is recognized to be a most important field among fields of Alzheimer's disease studies and its future prospects will be expected. Approaches to development of biological diagnosis markers up to now are divided into those based on constitute components of characteristic pathological changes of Alzheimer's disease such as senile plaque and neurofibril change, and an approach based on other measures. Examples of the former include cerebrospinal fluid tau protein, Aβ and its precursor, βAPP. Examples of the latter include mydriasis test with cholilytic drug, Apo E and other genes relating to Alzheimer's disease. However, no good results have been obtained.

Page 7, please amend the paragraph on page 7 as follows:

Serine proteases are also considered to play an important role in cancer cells. The reason why extermination of cancer by surgical treatment or topical irradiation of

radioactive ray is difficult is the metastatic capability of cancer. To spread solid tumor cells in a body, they loosen their adhesion to original adjacent cells, followed by separating from original tissue, passing through other tissues to reach the blood vessels or lymph nodes, entering into the circulatory system through stratum basal and endothelial layer of the vessel, leave from the circulatory system at somewhere in the body, and surviving and proliferating in a new environment. While adhesion to adjacent epidermal cells is lost when expression of cadherin which is an intercellular adhesive molecule of epithelium is stopped, to break through tissues is considered to depend on proteolytic enzymes which decompose an extracellular matrix.

Page 8, please amend the first paragraph as follows:

As enzymes which decompose the matrix, mainly, metal proteases (Rha, S. Y. et al., Breast Cancer Research Treatment, 43, 175, 1997) and serine proteases are known. They cooperate to decompose matrix proteins such as collagen, laminin and fibronectin. Among the serine proteases known to be concerned in decomposition of the matrix, in particular, there is urokinase type plasminogen activator (U-PA). U-PA has a role as a trigger specific for a protein decomposition chain reaction. Its direct target is plasminogen. It is present in blood abundantly and is a precursor of an inactive

serine protease which accumulates in reconstructed sites of tissues such as injured sites and tumors as well as inflammatory sites. In addition, as proteases which are concerned in metastasis and infiltration of cancers, for example, a tissue factor, lysosomal type hydrolase and collagenase have been known.

Page 8, please amend the second paragraph as follows:

At present, cancer is the top cause of death in Japan and more than 200,000 people die per year. Accordingly, specific substances which can be used as markers for diagnosis and therapy or prophylaxis of cancer are studied intensively. Such specific substances are referred to as tumor markers or tumor marker relating biomarkers. They are utilized in aid of diagnosis before treatment of cancer, for presuming carcinogenic organ and pathological tissue type, for monitoring effect of treatment, for finding recurrence early, for presuming prognosis, and the like. At present, tumor markers are essential in clinical analyses. Among them, alpha fetoprotein (AFP) which has high specificity to hepatocellular carcinoma and yolk sac tumor (Taketa K. et al., Tumour Biol., 9, 110, 1988), and carcinoembronic antigen (CEA) are used worldwide. In the future, tumor markers will be required more and more, and it is desired to develop, for example, organ

specific markers and tumor cell specific markers which are highly reliable serologic diagnosis of cancer. Up to now, humunglandular kallikrein (hK2) which is a serine protease expressed at human prostatic epithelial cells has been reported as a marker for prostatic cancer. And, hK2 has 78% homology with the sequence of prostatic specific antigen (PSA) and PSA is also used widely as a biochemical marker of prostatic cancer (Mikolajczyk, S. d. et al., Prostate, 34, 44, 1998; Pannek, J. et al., Oncology, 11, 1273, 1997; Chu, T. M. et al., Tumour Biology, 18, 123, 1997; Hsieh, M. et al., Cancer Res., 57, 2651, 1997). Further, hK2 is reported to be useful as a marker for not only prostatic cancer but also stomach cancer (Cho, J. Y. et al.. Cancer, 79, 878, 1997). Moreover, CYFRA (CYFRA 211) for measuring cytokeratin 19 fragment in serum is reported to be useful for lung cancer (Sugiyama, Y. et al., Japan J. Cancer Res., 85, 1178, 1994). Gastrin release peptide precursor (ProGRP) is reported to be useful as a tumor marker (Yamaguchi, K. et al., Japan, J. Cancer Res., 86, 698, 1995).

Page 10, please amend the second paragraph as follows:

Under these circumstances, the present inventors have succeeded in cloning cDNA encoding novel human and mouse serine proteases.

Page 10, please amend the third paragraph as follows:

In summary, the 1st feature of the present invention is the amino acid sequences of biologically active mature serine proteases BSSP2 and nucleotide sequences encoding the amino acid sequences.

Page 18, please amend the second paragraph as follows:

In case of northern blotting analysis, mBSSP2 shows the expression in the head of a 15-20 days mouse fetus, and in the lung, prostate and testicle of a 3 month-old mouse. hBSSP2 shows the expression in brain, skeletal muscle and liver (see Figs. 1, 2 and 5). In case of RT-PCR analysis, mBSSP2 shows the expression in the brain and testicle of a 12 day-old mouse, and hBSSP2 shows the expression in the brain and skeletal muscle. Then, the novel proteases of the present invention are presumed to play various roles in the brain, prostate, lung, testicle, skeletal muscle and liver. For example, in the brain, there is a possibility that they can be used for treatment and diagnosis of brain diseases such as Alzheimer's disease (AD), epilepsy, brain tumor and the like. Further, in other tissues, there is a possibility that BSSP2 of the present invention and a gene encoding it can be used for treatment and diagnosis of various diseases such as

cancer, inflammation, infertility, prostatomegaly and the like. Further, it is presumed they may have a certain influence on blood coagulation, fibrinolysis and complement systems. Furthermore, there is a possibility that inhibitors of serine proteases can be used for treatment and diagnosis of Alzheimer's disease, epilepsy, cancer, inflammation, infertility, prostatomegaly and the like.

Page 22, please amend the paragraph on page 22 as follows:

In general, many genes of eucaryotes exhibit polymorphism and, sometimes, one or more amino acids are substituted by this phenomenon. Further, even in such a case, sometimes, a protein maintains its activity. Then, the present invention includes a gene encoding a protein obtained by modifying a gene encoding any one of the amino acid sequences represented by SEQ ID NOS: 2, 4, 6, 8 and 10, artificially, in so far as the protein has the characteristic function of the gene of the present invention. Further, the present invention includes a protein which is a modification of any one of amino acid sequences represented by SEQ ID NOS: 2, 4, 6, 8 and 10 in so far as the protein has the characteristics of the present invention. Modification is understood to include substitution, deletion, addition and/or insertion. In particular, the present inventors have shown

that, even when several amino acids are added to or deleted from the N-terminus amino acid of the BSSP2 mature protein represented by SEQ ID NO: 2, the resultant sequence maintains its activity.

Page 23, please amend the first paragraph as follows:

That is, the present invention includes a protein comprising any one of the amino acid sequences described in SEQ ID NOS: 2, 4, 6, 8 and 10; an amino acid sequence encoded by any one of the nucleotide sequences represented by SEQ ID NOS: 1, 3, 5, 7 and 9; or one of these amino acid sequences wherein one to several amino acids have been substituted, deleted, added and/or inserted, and belonging to serine protease family.

Page 23, please amend the second paragraph as follows:

Each codon for the desired amino acid itself has been known and can be selected freely. For example, codons can be determined according to a conventional manner by taking into consideration the frequency of use of codons in a host to be utilized (Grantham, R. et al., Nucleic Acids Res., 9, r43, 1989). Therefore, the present invention also includes a nucleotide sequence appropriately modified by

taking into consideration the degeneracy of a codon. Further, these nucleotide sequences can be modified by a site directed mutagenesis using a primer composed of a synthetic oligonucleotide encodinq the desired modification (Mark, D. F. et al., Proc. Natl. Acad. Sci. USA., 81, 5662, 1984), or the like.

Page 23, please amend the third paragraph as follows:

Furthermore, the DNA of the present invention includes DNA which is hybridizable to any one of the nucleotide sequences described in SEQ ID NOS: 1, 3, 5, 7 and 9 or nucleotide sequences complementary to these nucleotide sequences in so far as the protein encoded by the nucleotide sequence has the same properties as those of the BSSP2 of the present invention. It is considered that many of the sequences which are hybridizable to a given sequence under stringent conditions have a similar activity to that of a protein encoded by the given sequence. The stringent conditions according to the present invention includes, for example, incubation in a solution containing 5 x SSC, 5% Denhardt's solution (0.1% BSA, 0.1% Ficol 1400, 0.1% PVP), 0.5% SDS and 20 µg/ml denatured salmon sperm DNA at 37°C overnight, followed by washing with 2 x SSC containing 0.1% SDS at room temperature. Instead of SSC, SSPE can be

appropriately used.

Page 25, please amend the second paragraph as follows:

The present invention also relates to a vector comprising the nucleotide sequence represented by SEQ ID NO: 1 or a nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 2; the nucleotide sequence represented by SEQ ID NO: 3 or a nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 4; the nucleotide sequence represented by SEQ ID NO: 5 or a nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 6; the nucleotide sequence represented by SEQ ID NO: 7 or a nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 8; or the nucleotide sequence represented by SEQ ID NO: 9 or a nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 10; or a nucleotide sequence similar to them. A nucleotide sequence similar to a given nucleotide sequence used herein means a nucleotide sequence which is hybridizable to the given nucleotide sequence or its complementary nucleotide sequence under the above-described stringent conditions and which encodes a protein having the same properties as those of the protein encoded by the nucleotide sequence.

Page 28, please amend the first paragraph as follows:

The animal cells and insect cells used herein include cells derived from human beings and cells derived from flies or silk worms. For example there are CHO cells, COS cells, BHK cells, Vero cells, myeloma cells, HEK293 cells, HeLa cells, Jurkat cells, mouse L cells, mouse C127 cells, 10 mouse FM3A cells, mouse fibroblast, osteablast, cartilage cells, S2, Sf9, Sf21, High Five™ (registered trade mark) cells and the like.

Page 28, please amend the second paragraph as follows:

The protein of the present invention as such can be expressed as a recombinant fused protein so as to facilitate isolation, purification and recognition. The recombinant fused protein used herein means a protein expressed as an adduct wherein a suitable peptide chain is added to the N-terminus and/or C-terminus of the desired protein expressed by a nucleotide sequence encoding the desired protein. The recombinant protein used herein means that obtained by integrating a nucleotide sequence encoding the desired protein in the expression vector of the present invention and cut off an amino acid sequence which derived from nucleic acids other than those encoding the desired protein from the expressed recombinant

fused protein, and is substantially the same as the protein of the present invention.

Page 29, please amend the first paragraph as follows:

Introduction of the above vector into host cells can be carried out by, for example, transfection according to the lipopolyamine method, DEAE-dextran method, Hanahan method, lipofectin method or calcium phosphate method, microinjection, eletroporation and the like.

Page 33, please amend the first paragraph as follows:

For obtaining fertilized egg cells efficiently, ovulation may be induced with gonadotropin or the like. Fertilized egg cells are recovered and a gene in an injection pipette is injected into male pronucleus of the egg cells by microinjection. For returning the injected egg cells to a fallopian tube, an animal (false pregnancy female mouse, etc.) is provided and about 10 to 15 eggs/mice are transplanted. Then, genomic DNA is extracted from the end part of the tail to confirm whether the transgene is introduced into newborn mouse or not. This confirmation can be carried out by detection of the transgene with southern blot technique or PCR technique, or by positive cloning

wherein a marker gene, which is activated only when homologous recombination is caused, has been introduced. Further, transcribed products derived from the transgene are detected by northern blot technique or RT-PCR technique to confirm expression of the transgene. Or, western blotting can be carried out with a specific antibody to a protein.

Page 33, please amend the second paragraph as follows:

The knockout mouse of the present invention is treated so that the function of mBSSP2 gene is lost. A knockout mouse means a transgenic mouse in which any one of its genes is destroyed by homologous recombination technique so that its function is deficient. A knockout mouse can be created by carrying out homologous recombination with ES cells and selecting embryonic stem cells wherein either of allele genes are modified or destroyed. For example, embryonic stem cells whose genes are manipulated at the blastocyte or morula stage of fertilized eggs are injected to obtain a chimeric mouse wherein cells derived from the embryonic stem cells are mixed with those derived from the embryo. The chimeric mouse (chimeric means a single individual formed by somatic cells based on two or more fertilized eggs) can be mated with a normal mouse to create a heterozygote mouse wherein all of the allele genes have been

modified or destroyed. Further, a homozygote mouse can be created by mating heterozygote mice.

Page 37, please amend the paragraph on page 37 as follows:

Examples of myeloma cells include X-63Ag8, NS-1, P3U1, SP2/0, AP-1 and the like with SP2/0 being preferred. The preferred ratio of the number of the antibody producer cells (spleen cells) : the number of spleen cells are 1 : 20 to 20 : 1. PEG (preferably PEG 1000 to PEG 6000) is added at a concentration of about 10 to 80% and the mixture is incubated at 20 to 40°C, preferably 30 to 37°C for 1 to 10 minutes to carry out the cell fusion efficiently. Screening of anti-hBSSP2 or mBSSP2 antibody producer hybridomas can be carried out by various methods. For example, a supernatant of a hybridoma culture is added to a solid phase to which hBSSP2 or mBSSP2 antigen is adsorbed directly or together with a carrier (e.g., microplate), followed by addition of an anti-immunoglobulin antibody (in case that the cells used in cell fusion are those of a mouse, anti-mouse immunoglobulin antibody is used) or protein A to detect the anti-hBSSP2 or mBSSP2 monoclonal antibody attached to the solid phase. Or, a supernatant of a hybridoma culture is added to a solid phase to which an anti-immunoglobulin antibody or protein A is adsorbed, followed by addition of hBSSP2 or mBSSP2 labeled with a radioactive substance, an enzyme, etc., to detect the

anti-hBSSP2 or mBSSP2 monoclonal antibody attached to the solid phase.

Page 38, please amend the first paragraph as follows:

Selection and cloning of the anti-hBSSP or mBSSP monoclonal antibody can be carried out according to a per se known method or its modification. Normally, a HAT (hypoxanthine, aminopterin, thymidine)-added medium for culturing animal cells is used. Any culture medium can be used for selection, cloning and growing up in so far as the hybridoma can grow. For example, there can be used RPMI culture medium containing 1 to 20%, preferably 10 to 20% fetal bovine serum, a serum-free medium for culturing hybridomas. Preferably, the culture is carried out at a temperature of about 37°C. Normally, the culture time is 5 days to 3 weeks, preferably 1 week to 2 weeks. Normally, the culture is carried out under 5% CO_2. The antibody titer of a supernatant of a hybridoma culture can be measured according to the same manner as that of the above-described measurement of anti-BSSP2 antibody titer in an antiserum. That is, examples of the measurement to be used include radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), FIA (fluorescence immunoassay), plaque assay, agglutination reaction method, and the like. Among them, ELISA as shown below is preferred.

Page 39, please amend the paragraph on page 39 as follows:

Normally, cloning is carried out by a per se known method such as semi-solid agar method, limiting dilution method and the like. Specifically, after confirming a well in which the desired antibody is produced by the above-described method, cloning is carried out to obtain a single clone. For cloning, it is preferred to employ limiting dilution method wherein hybridoma cells are diluted so that one colony is formed per one well of a culture plate. For cloning by limiting dilution method, feeder cells can be used, or a cell growth factor such as interleukin 6, etc. can be added to improve colony forming capability. In addition, cloning can be carried out by using FACS and single cell manipulation method. The cloned hybridoma is preferably cultured in a serum-free culture medium and an optimal amount of an antibody is added to its supernatant. The single hybridoma thus obtained can be cultured in a large amount by using a flask or a cell culture device, or cultured in the abdominal cavity of an animal (J. Immunol. Meth., 53, 313, 1982) to obtain a monoclonal antibody. When culturing in a flask, there can be used a cell culture medium (e.g., IMDM, DMEM, RPMI1640, etc.) containing 0 to 20% of FCS. When culturing in the abdominal cavity of an animal, the animal to be used is preferably the same species or the same line as that from which the myeloma cells used in the cell fusion are derived, a thymus

deficient nude mouse or the like, and the hybridoma is transplanted after administration of a mineral oil such as pristane, etc. After 1 to 2 weeks, myeloma cells are proliferated in the abdominal cavity to obtain ascites containing a monoclonal antibody.

Page 42, please amend the paragraph on page 42 as follows:

The polyclonal antibody of the present invention can be produced according to a per se known method or its modification. For example, an immunogen (protein antigen) per se or a complex thereof with a carrier protein is prepared and, according to the same manner as that in the above monoclonal antibody production, a warm-blooded animal is immunized. A material containing an antibody against the protein of the present invention or its fragment is collected from the immunized animal and the antibody is separated and purified to obtain the desired antibody. As for a complex of an immunogen and a carrier protein for immunizing a warm-blooded animal, the kind of a carrier protein and the mixing ratio of a carrier and a hapten are not specifically limited in so far as an antibody against hapten immunized by cross-linking with the carrier is efficiently produced. For example, there can be used about 0.1 to 20, preferably about 1 to 5 parts by weight of bovine serum albumin, bovine cycloglobulin, hemocyanin, etc. coupled with one part by

weight of a hapten. For coupling a carrier and a hapten, various condensing agents can be used. Examples thereof include glutaraldehyde, carbodiimide or maleimide active ester, active ester agents having thiol group or dithiopyridyl group, and the like. The condensed product is administered as such or together with a carrier or diluent to a site of a warm-blooded animal where an antibody can be produced. For enhancing the antibody production, upon administration, Freund's complete adjuvant or Freund's incomplete adjuvant may be administered. Normally, the administration is carried out once every 2 to 6 weeks, 3 to 10 times in all. The polyclonal antibody can be collected from blood, ascites, or the like, preferably blood of the immunized animal. The polyclonal antibody titer in an antiserum can be measured according to the same manner as measurement of the above monoclonal antibody titer in the antiserum. Separation and purification of the polyclonal antibody, like the above monoclonal antibody, can be carried out according to the same manner as those of immunoglobulins.

Page 44, please amend the second paragraph as follows:

As a sandwich method for determining hBSSP2 or mBSSP2 or a fragment thereof, there can be used a two step method, a one step method and the like. In the two step method, first, the immobilized antibody is reacted with hBSSP2 or mBSSP2 or a fragment thereof and then unreacted materials are completely

removed by washing, followed by addition of the labeled antibody to form immobilized antibody-hBSSP2 or mBSSP2-labeled antibody. In the one step method, the immobilized antibody, labeled antibody and hBSSP2 or mBSSP2 or a fragment thereof are added at the same time.

Page 45, please amend the second paragraph as follows:

For immobilization of the antibody, a known chemical bonding method or a physical adsorption can be used. Examples of a chemical bonding method include a method using glutaraldehyde; maleimide method using N-succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate, N-succinimdyl-2-maleimide acetate or the like; carbodiimide method using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride; or the like. In addition, there are maleimidobenzoyl-N-hydroxysuccinimide ester method, N-succinimidyl-3-(2-pyridylthio)propionic acid method, bisdiazobenzidine method, and dipalmityllysine method. Or, it is possible to capture a complex formed beforehand by reacting a material to be tested with two antibodies, whose epitopes are different, with an immobilized a 3rd antibody against the antibody.

Page 46, please amend the paragraph on page 46 as follows:

For labeling, it is preferred to use an enzyme, fluorescent substance, luminous substance, radioactive substance, metal chelate, or the like. Examples of the enzyme include peroxidase, alkaline phosphatase, β-D-galactosidase, malate dehydrogenase, *Staphylococcus* nuclease, δ-5-steroidisomerase, α-glycerol phosphate dehydrogenase, triose phosphate isomerase, horseradish peroxidase, asparaginase, glucose oxidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase, acetylcholinesterase and the like. Examples of the fluorescent substance include fluorescein isothiocyanate, phycobiliprotein, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, o-phthalaldehyde, and the like. Examples of the luminous substance include isoluminol, lucigenin, luminol, aromatic acridinium ester, imidazole, acrdinium salt and its modified ester, luciferin, luciferase, aequorin and the like. Examples of the radioactive substance include ^{125}I, ^{127}I, ^{131}I, ^{14}C, ^{3}H, ^{32}P, ^{35}S and the like. The labeling material is not limited to them and any material which can be used for immunological determination can be used. Further, a low molecular weight hapten such as biotin, dinitrophenyl, pyridoxal or fluorescamine may be attached to the antibody. Preferably, horseradish peroxidase is used as a labeling enzyme. This enzyme can be reacted with various substrates and can readily be attached to

the antibody by periodate method.

Page 47, please amend the first paragraph as follows:

When an enzyme is used as a labeling material, a substrate and, if necessary, a coloring enzyme is used for measuring its activity. In case of using peroxidase as the enzyme, H_2O_2 is used as a substrate and, as a coloring agent, there can be used 2,2'-azino-di-[3-ethylbenzthiazoline sulfonic acid] ammonium salt (ABTS), 5'-aminosalicylic acid, o-phenylenediamine, 4-aminoantipyrine, 3,3',5,5'-tetramethylbenzidine and the like. In case of using alkaline phosphatase as the enzyme, o-nitrophenylphosphate, p-nitrophenylphosphoric acid, or the like can be used as a substrate. In case of using β-D-galactosidase as the enzyme, fluorescein-d-(β-D-galactopyranoside), 4-methylumbelliphenyl-β-D-galactopyranoside, or the like can be used as a substrate. The present invention also includes a kit comprising the above monoclonal antibody, polyclonal antibody and reagents.

Page 47, please amend the second paragraph as follows:

As a cross-linking agent, a known cross-linking agent such as N,N'-o-phenylenedimaleimide, 4-(N-maleimidomethyl)cyclohexanoate-N-succinimide ester, 6-maleimidohexanoate-N-succineimide ester, 4,4'-dithiopyridine or the like can be utilized. The reaction of these cross-linking

agents with enzymes and antibodies can be carried out by a known method according to properties of a particular cross-linking agent. Further, as the antibody, a fragment thereof, for example, Fab', Fab, F(b'2) can be used as the case may be. A labeled enzyme can be obtained by the same treatment regardless of whether the antibody is polyclonal or monoclonal. When the above labeled enzyme obtained by using a cross-linking agent is purified by a known method such as affinity chromatography or the like, an immunoassay system having more higher sensitivity can be obtained. The enzyme labeled and purified antibody is stored in a dark cold place with addition of a stabilizer such as thimerosal, glycerin or after lyophilization.

Page 49, please amend the paragraph on page 49 as follows:

The cloning was carried out by PCR using a mouse brain cDNA library (Clontech) as a template and nucleotide sequences corresponding to an amino acid sequence common to serine proteases represented by

Primer 1: GTG CTC ACN GCN GCB CAY TG (SEQ ID NO: 20)

Primer 2: CCV CTR WSD CCN CCN GGC GA (SEQ ID NO: 21)

as primers. Namely, 5 µl of the template, 5 µl of 10 x ExTaq buffer, 5 µl of dNTP, 10 pmol of each of the above primers and 0.5 µl of ExTaq (TAKARA) were added and the total volume was adjusted to 50 µl with sterilized water. PCR was carried out by

repeating a cycle of heating at 94°C for 0.5 minute, at 55°C for 0.5 minute and then at 72°C for 1 minute, 30 times. The PCR product was mixed with pCR II-TOPO vector attached to TOPO TA cloning kit (Invitrogen) and the mixture was allowed to stand at room temperature for 5 minutes. Then, according to a conventional manner, *E. coli* Top 10 attached to the kit was transformed and applied to a LB (Amp^+) plate (containing 100 µg/ml of ampicillin). According to a conventional manner, a plasmid was extracted from each colony obtained and its nucleotide sequence was determined by cycle sequencing method with a fluorescence sequencer (ABI). Homology of the sequence of each clone was examined by means of GenBank. Regarding an unknown sequence, i.e., BSSP2 gene, the full length cDNA was obtained by 5' RACE and 3' RACE and, according to the same manner as described above, the nucleotide sequence was determined. Namely, BSSP2 clone specific primers, GSP1 primers [mBSSP2.2 (SEQ ID NO: 27) or mBSSP2.0 (SEQ ID NO: 22)] and GSP2 primers [mBSSP2R2 (SEQ ID NO: 28) or mBSSP2.1 (SEQ ID NO: 23)] were prepared. PCR was carried out by using mouse brain Marathon-Ready cDNA (Clontech), AP1 primer attached to this reagent and either of the above GSP1 primers and heating at 94°C for 2 minutes once and repeating a cycle of heating at 94°C for 30 seconds, at 60°C for 30 seconds and then at 72°C for 30 seconds 35 times. Then, 5 µl of the PCR product diluted to 1/100, 5 µl of 10 x buffer, 5 µl of dNTP, 10 pmol of either of 10 µM of the

above GSP2 primer, 10 pmol of AP2 primer attached to the above reagent and 0.5 unit of ExTaq were admixed and adjusted to 50 µl with sterilized water. Then, according to the same manner as the above, PCR was carried out. The PCR product was cloned by the above TOPO TA cloning kit and sequenced to obtain the upstream and downstream regions of the above clone. At this time, as for a clone which seemed not to cover the full length of a protein, the specific primers shown hereinafter were prepared based on the newly found nucleotide sequence. Further, based on this sequence, the primers capable of amplifying ORF as shown hereinafter [mBSSPF7 (SEQ ID NO: 26), mBSSP2R/E (SEQ ID NO: 29)] were prepared and PCR carried out using mouse brain Marathon-ready cDNA as a template to confirm that these clones were identical. This was cloned into pCR II-TOPO vector attached to TOPO TA cloning kit to obtain the plasmid pCR II/mBSSP2 containing the full length cDNA clone. The nucleotide sequence of DNA contained in this plasmid is shown in SEQ ID NO: 7 and the amino acid sequence of mSSP2 protein deduced from the nucleotide sequence is shown in SEQ ID NO: 8. Further, two different types of clones were obtained. The nucleotide sequences of these DNA are shown in SEQ ID NOS: 3 and 5, respectively. The amino acid sequences of mBSSP2 proteins deduced from these nucleotide sequences are shown in SEQ ID NOS: 4 and 6. These novel proteases are divided into types 1, 2 and 3. Type 1 is composed of 273 amino acids, type 2 is composed of 311 amino acids and

type 3 is composed of 445 amino acids. These amino acid sequences contained the common amino acid sequence composed of 238 amino acids whose N-terminus side started with Ile-Val-Gly-Gly-Gln-Ala-Val as the mature serine protease. Further, in the amino acid sequence of the mature serine protease, a consensus sequence having a serine protease activity was contained. Furthermore, since there were two or more amino acid sequences specific for a sugar chain bonding site, it was presumed that the amino acid sequence had at least two sugar chains.

Page 53, please amend the paragraph on page 53 as follows:

As seen from Figs. 1 and 2, in the case of northern blotting analysis, the expression of mBSSP2 was recognized in the head of 15th to 20th day fetuses of mice and, as to the 3-month-old mice, the expression was recognized in the prostate and testicle. Further, according to the results of RT-PCR, the expression was recognized in the head of 12-day-old mice and the testicle of 3-month-old mice.

IN THE CLAIMS

Cancel claims 1-19 and 37-40 and enter the following new claims:

41. (New) A protein selected from the group consisting of:

(a) a protein having the amino acid sequence of 238 amino acids represented by SEQ ID NO: 2;

(b) a protein having an amino acid sequence derived from the amino acid sequence represented by SEQ ID NO: 2 by deletion, substitution or addition of one to several amino acids and having the same property as that of the protein having the amino acid sequence represented by SEQ ID NO: 2;

(c) a protein having the amino acid sequence of 273 amino acids represented by SEQ ID NO: 4;

(d) a protein having an amino acid sequence derived from the amino acid sequence represented by SEQ ID NO: 4 by deletion, substitution or addition of one to several amino acids and having the same property as that of the protein having the amino acid sequence represented by SEQ ID NO: 4;

(e) a protein having the amino acid sequence of 311 amino acids represented by SEQ ID NO: 6;

(f) a protein having an amino acid sequence derived from the amino acid sequence represented by SEQ ID NO: 6 by deletion, substitution or addition of one to several amino acids and having the same property as that of the protein having the amino acid sequence represented by SEQ ID NO: 6;

(g) a protein having the amino acid sequence of 455 amino acids represented by SEQ ID NO: 8;

(h) a protein having an amino acid sequence derived from the amino acid sequence represented by SEQ ID NO: 8 by

deletion, substitution or addition of one to several amino acids and having the same property as that of the protein having the amino acid sequence represented by SEQ ID NO: 8;

(i) a protein having the amino acid sequence of 240 amino acids represented by the 1st to 240th amino acids of SEQ ID NO: 10;

(j) a protein having an amino acid sequence derived from the amino acid sequence represented by the 1st to 240th amino acids of SEQ ID NO: 10 by deletion, substitution or addition of one to several amino acids and having the same property as that of the protein having the amino acid sequence represented by the 1st to 240th amino acids of SEQ ID NO: 10;

(k) a protein having the amino acid sequence of 457 amino acids represented by the -217th to 240th amino acids of SEQ ID NO: 10;

(l) a protein having an amino acid sequence derived from the amino acid sequence represented by the -217th to 240th amino acids of SEQ ID NO: 10 by deletion, substitution or addition of one to several amino acids and having the same property as that of the protein having the amino acid sequence represented by the -217th to 240th amino acids of SEQ ID NO: 10;

(m) a protein having the amino acid sequence of 217 amino acids represented by the -217th to -1st amino acids of SEQ ID NO: 10;

(n) a protein having an amino acid sequence derived from the amino acid sequence represented by the -217th to -1st amino acids of SEQ ID NO: 10 by deletion, substitution or addition of one to several amino acids and having the same property as that of the protein having the amino acid sequence represented by the -217th to -1st amino acids of SEQ ID NO: 10; and

(o) a modified derivative or fragment of these proteins (a) to (n).

42. (New) A nucleotide sequence selected from the group consisting of:

(aa) a nucleotide sequence represented by the 1st to 714th bases of SEQ ID NO: 1;

(bb) a nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 2;

(cc) a nucleotide sequence hybridizable with a nucleotide sequence which is complementary to the above nucleotide sequence (aa) or (bb) under stringent conditions and encoding a protein having the same property as that of the protein having the amino acid sequence represented by SEQ ID NO: 2;

(dd) a nucleotide sequence represented by the 247th to 1065th bases of SEQ ID NO: 3;

(ee) a nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 4;

(ff) a nucleotide sequence hybridizable with a nucleotide sequence which is complementary to the above nucleotide sequence (dd) or (ee) under stringent conditions and encoding a protein having the same property as that of the protein having the amino acid sequence represented by SEQ ID NO: 4;

(gg) a nucleotide sequence represented by the 516th to 1448th bases of SEQ ID NO: 5;

(hh) a nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 6;

(ii) a nucleotide sequence hybridizable with a nucleotide sequence which is complementary to the above nucleotide sequence (gg) or (hh) under stringent conditions and encoding a protein having the same property as that of the protein having the amino acid sequence represented by SEQ ID NO: 6;

(jj) a nucleotide sequence represented by the 116th to 1450th bases of SEQ ID NO: 7;

(kk) a nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 8;

(ll) a nucleotide sequence hybridizable with a nucleotide sequence which is complementary to the above nucleotide sequence (jj) or (kk) under stringent conditions and

encoding a protein having the same property as that of the protein having the amino acid sequence represented by SEQ ID NO: 8;

(mm) a nucleotide sequence represented by the 807th to 1526th bases of SEQ ID NO: 9;

(nn) a nucleotide sequence encoding the amino acid sequence represented by the 1st to 240th amino acids of SEQ ID NO: 10;

(oo) a nucleotide sequence hybridizable with a nucleotide sequence which is complementary to the above nucleotide sequence (mm) or (nn) under stringent conditions and encoding a protein having the same property as that of the protein having the amino acid sequence represented by the 1st to 240th amino acids of SEQ ID NO: 10;

(pp) a nucleotide sequence represented by the 156th to 1526th bases of SEQ ID NO: 9;

(qq) a nucleotide sequence encoding the amino acid sequence represented by the -217th to 240th amino acids of SEQ ID NO: 10;

(rr) a nucleotide sequence hybridizable with a nucleotide sequence which is complementary to the above nucleotide sequence (pp) or (qq) under stringent conditions and encoding a protein having the same property as that of the protein having the amino acid sequence represented by the -217th to 240th amino acids of SEQ ID NO: 10;

(ss) a nucleotide sequence represented by the 156th to 806th bases of SEQ ID NO: 9;

(tt) a nucleotide sequence encoding the amino acid sequence represented by the -217th to -1st amino acids of SEQ ID NO: 10;

(uu) a nucleotide sequence hybridizable with a nucleotide sequence which is complementary to the above nucleotide sequence (ss) or (tt) under stringent conditions and encoding a protein having the same property as that of the protein having the amino acid sequence represented by the -217th to -1st amino acids of SEQ ID NO: 10;

(vv) a nucleotide sequence represented by SEQ ID NO: 1;

(ww) a nucleotide sequence hybridizable with a nucleotide sequence which is complementary to the above nucleotide sequence (vv) under stringent conditions and encoding a protein having the same property as that of the protein encoded by the nucleotide sequence represented by SEQ ID NO: 1;

(xx) a nucleotide sequence represented by SEQ ID NO: 3;

(yy) a nucleotide sequence hybridizable with a nucleotide sequence which is complementary to the above nucleotide sequence (xx) under stringent conditions and encoding a protein having the same property as that of the protein encoded by the nucleotide sequence represented by SEQ ID NO: 3;

(zz) a nucleotide sequence represented by SEQ ID NO: 5;

(aaa) a nucleotide sequence hybridizable with a nucleotide sequence which is complementary to the above nucleotide sequence (zz) under stringent conditions and encoding a protein having the same property as that of the protein encoded by the nucleotide sequence represented by SEQ ID NO: 5;

(bbb) A nucleotide sequence represented by SEQ ID NO: 7;

(ccc) a nucleotide sequence hybridizable with a nucleotide sequence which is complementary to the above nucleotide sequence (bbb) under stringent conditions and encoding a protein having the same property as that of the protein encoded by the nucleotide sequence represented by SEQ ID NO: 7;

(ddd) a nucleotide sequence represented by SEQ ID NO: 9;

(eee) a nucleotide sequence hybridizable with a nucleotide sequence which is complementary to the above nucleotide sequence (ddd) under stringent conditions and encoding a protein having the same property as that of the protein encoded by the nucleotide sequence represented by SEQ ID NO: 9; and

(fff) a fragment of these nucleotide sequences (aa) to (eee).

43. (New) The process according to claim 23, wherein

the cells are *E. coli* cells, animal cells or insect cells.

44. (New) The method according to claim 33, wherein the specimen is a body fluid.

45. (New) The method according to claim 34, wherein the specimen is a body fluid.

46. (New) A method for screening for an inhibitor of serine protease comprising comparing the enzyme activity of the protein according to claim 41 upon bringing the protein into contact with a candidate compound with the enzyme activity of the protein without contact with the candidate compound.

47. (New) A pharmaceutical composition comprising the protein according to claim 41.

48. (New) A method for detecting a diagnostic marker for diseases in tissues comprising the protein according to claim 41, which comprises using an antibody against the protein according to claim 41.

49. (New) The method according to claim 48, wherein the marker is used for diagnosis of a cancer.

50. (New) A method for diagnosing Alzheimer's disease or epilepsy in the brain comprising using the marker according to claim 36.

51. (New) A method for diagnosing cancer or inflammation of the brain, prostate or testicle, comprising using the marker according to claim 36.

52. (New) A method for diagnosing sterility in semen or sperm comprising using the marker according to claim 36.

53. (New) A method for diagnosing prostatic hypertrophy comprising using the marker according to claim 36.

Please amend claim 20 as follows:

20. (Amended) A vector comprising the nucleotide sequence according to claim 42.

Please amend claim 21 as follows:

21. (Amended) Transformed cells having the nucleotide sequence according to claim 42 in an expressible state.

Please amend claim 22 as follows:

22. (Amended) A process for producing a protein which comprises culturing cells transformed with the nucleotide sequence (aa) to (ll), (vv) to (zz), (aaa), (bbb) or (ccc) of claim 42, and collecting mBSSP2 produced.

Please amend claim 23 as follows:

23. (Amended) A process for producing a protein which comprises culturing cells transformed with the nucleotide sequence (mm) to (uu), (ddd) or (eee) of claim 42, and collecting hBSSP2 produced.

Please amend claim 24 as follows:

24. (Amended) The process according to claim 22, wherein the cells are *E. coli* cells, animal cells or insect cells.

Please amend claim 29 as follows:

29. (Amended) An antibody against the protein according to claim 41 or a fragment thereof.

Please amend claim 31 as follows:

31. (Amended) A process for producing a monoclonal antibody against the protein according to claim 41 or a fragment thereof which comprises administering the protein according to

claim 41 or a fragment thereof to a warm-blooded animal other than a human being, selecting the animal whose antibody titer is recognized, collecting its spleen or lymph node, fusing the antibody producing cells contained therein with myeloma cells to prepare a monoclonal antibody producing hybridoma.

Please amend claim 32 as follows:

32. (Amended) A method for determining the protein according to claim 41 or a fragment thereof in a specimen which is based on immunological binding of an antigen against the protein or a fragment thereof to the protein or a fragment thereof in the specimen.

Please amend claim 33 as follows:

33. (Amended) A method for determining hBSSP2 or a fragment thereof in a specimen which comprises reacting a monoclonal antibody or a polyclonal antibody against the protein (i), (j), (k), (l), (m) or (n) of claim 41 or a modified derivative or fragment thereof and a labeled antibody with hBSSP2 or a fragment thereof in the specimen to detect a sandwich complex produced.

Please amend claim 34 as follows:

34. (Amended) A method for determining hBSSP2 or a fragment thereof in a specimen which comprises reacting a

monoclonal antibody or a polyclonal antibody against the protein (i), (j), (k), (l), (m) or (n) of claim 41 or a modified derivative thereof or a fragment thereof with labeled hBBSP2 and hBSSP2 or a fragment thereof in the specimen competitively to detect an amount of hBSSP2 or a fragment thereof in the specimen based on an amount of the labeled hBBSP2 reacted with the antibody.

Please amend claim 35 as follows:

35. (Amended) The method according to claim 32, wherein the specimen is a body fluid.

Please amend claim 36 as follows:

36. (Amended) A diagnostic marker for diseases in tissues comprising the protein according to claim 41.

REMARKS

The present preliminary amendment is submitted in order to correct self-evident typographical and grammatical errors in the specification, as well as to define the invention better.

It is respectfully submitted that the claims are now in condition for examination, and prompt and favorable action is earnestly solicited.

Respectfully submitted,

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"Version with markings to show changes"

Page 1, please amend the second paragraph as follows:

In general, proteases are biosynthesized as inactive precursors. They undergo limited hydrolysis in molecules to ~~convert~~ be converted into activated type proteases. In so far as enzymes are proteases, they have an activity for hydrolyzing a peptide bond, while their ~~action modes are varied~~ actions vary according to kinds of proteases. According to a particular kind of catalytic site, proteases are divided into serine proteases, cysteine proteases, aspartate proteases, metal proteases and the like. Proteases of each kind have a variety of properties, ranging from a protease having general digestive properties to a protease having various regulatory domains and strict substrate specificity, thereby specifically hydrolyzing only characteristic proteins.

Page 2, please amend the paragraph on page 2 as follows:

Further, proteins undergo various types of processing even after translation to produce active proteins. In many secretory proteins, a protein ~~are~~is first synthesized on the ribosome in cytoplasm as an inactive precursor (pro-form) which comprises an active protein bearing at the N-terminus thereof a peptide of about 15 to 60 amino acids responsible for secretion (secretory signal). This peptide region is concerned with the

mechanism for passing through the cell membrane and is removed upon cleavage by a specific protease during the passage through the membrane, in almost all the cases, to produce the mature form. A secretory signal has a broad hydrophobic region comprising hydrophobic amino acids in the middle of the sequence, and basic amino acid residues at a site close to the N-terminus. A secretory signal is a synonym ~~of~~ for a signal peptide. In addition, in some proteins, a peptide moiety which functions as a secretory signal is further attached to the N-terminus of the inactive precursor (pro-form). Such a protein is called a prepro-protein (prepro-form).

Page 3, please amend the first paragraph as follows:

For example, trypsin is present ~~as~~ in the form of a prepro-form immediately after translation into amino acids. After being secreted from cells, it is present ~~as~~ in the form of a pro-form and is then converted into active trypsin in the duodenum upon limited hydrolysis by enteropeptidase or by trypsin itself.

Page 3, please amend the second paragraph as follows:

The optimal pH range of serine proteases is neutral to weak alkaline and, in general, many of them have a molecular

weight of about 30,000 or lower. All proteases ~~of~~ relating to blood coagulation, fibrinolysis and complement systems having a large molecular weight belong to the family of trypsin-like serine proteases. They have many regulator domains and form a protease cascade which is of very importance to reactions in a living body.

Page 4, please amend the first paragraph as follows:

Serine proteases expressed in a brain-nerve system such as neurosin are considered to play various roles in the brain-nerve system. Therefore, there is a possibility that isolation of a gene encoding a novel protease expressed in a brain-nerve system and production of a protein using the gene would be useful for diagnosis or ~~therapeutic~~ therapy of various diseases related to the brain-nerve system.

Page 4, please amend the second paragraph as follows:

Nowadays, in general, clinical diagnosis of Alzheimer's disease is ~~conducted~~ based on the diagnosis standard of DSM-IIIR and NINCDS-ADRDA (Mckhann, G. et al., Neurology, 34. 939, 1994) or the diagnosis standard of DSM-IV (American Psychiatric Association; Diagnostic and statistical manuals of mental disorders, 4th ed., Washington DC, American Psychiatric Association, 1994). However, these standards are conditioned by a decline ~~of~~ in recognition functions which causes

a severe disability in ~~a~~ daily life or ~~a~~ social life. Then, it is pointed out that the diagnosis is less ~~scientific objectivity~~ **than scientifically objective** because the diagnosis may be influenced by the level of an individual's social life and further the specialty and experience of a physician who diagnoses particular conditions. In addition, definite diagnosis of Alzheimer's disease is conducted by pathohistological analyses and, in this respect, substantial inconsistency between clinical diagnosis and autopsy diagnosis ~~is pointed out~~ exists.

Page 5, please amend the paragraph on page 5 as follows:

At present, image diagnosis is employed as a supplemental means in clinical diagnosis of Alzheimer's diagnosis and it is possible to analyze brain functions, for example, decline of metabolism and atrophy in specific sites such as hippocampus, parietal lobe of cerebral cortex and the like which are specific for Alzheimer's disease by PET and SPECT. However, to define Alzheimer's disease based on lowering of a blood flow from parietal lobe to temporal lobe is very dangerous. In addition, there is ~~few~~ a report showing that MRS ~~testicle~~ test **is** useful for patients with dementia including those of Alzheimer's disease. Further, although CT-MRI image diagnosis is used, a lesion of white matter such as atrophy of brain, PVL or the like is not specific for Alzheimer type dementia. Since

it has been reported that atrophy of brain proceeds ~~as getting older~~ with aging, the above observation is not necessarily found in Alzheimer type dementia. Furthermore, since an image obtained by MRI varies according to strength of a magnetic field, performance of ~~an~~ the apparatus and imaging conditions, numerical data ~~obtain~~ obtained in different facilities cannot be compared with each other except for atrophic change. In addition, there is a limit to image measurement. Further, enlargement of the ventricle can be recognized in vascular dementia cases and there are cases wherein atrophy of the hippocampus is observed after ischemia of the basilar artery.

Page 6, please amend the fourth paragraph as follows:

Further, data obtained in different facilities can be compared with each other by using the same diagnosis marker. Therefore, development of biological diagnosis markers is recognized to be a most important field among fields of Alzheimer's disease studies and its future prospects will be expected. Approaches to development of biological diagnosis markers up to now are divided into ~~that~~ those based on constitute components of characteristic pathological changes of Alzheimer's disease such as senile plaque and neurofibril change, and an approach based on other measures. Examples of the former include cerebrospinal fluid tau protein, Aβ and its precursor, βAPP.

Examples of the latter include mydriasis test with cholilytic drug, Apo E and other genes relating to Alzheimer's disease. However, no good results ~~are~~ have been obtained.

Page 7, please amend the paragraph on page 7 as follows:

Serine proteases are also considered to play an important role in cancer cells. The reason why extermination of cancer by surgical treatment or topical irradiation of radioactive ray is difficult is ~~metastasis~~ the metastatic capability of cancer. ~~For~~ To spread ~~of~~ solid tumor cells in a body, they ~~should~~ loosen their adhesion to original adjacent cells, followed by separating from ~~an~~ original tissue, passing through other tissues to reach the blood ~~vessel~~ vessels or lymph ~~node~~ nodes, entering into the circulatory system through stratum basal and endothelial layer of the vessel, leave from the circulatory system at somewhere in the body, and surviving and proliferating in a new environment. While adhesion to adjacent epidermal cells is lost when expression of cadherin which is an intercellular adhesive molecule of epithelium is stopped, to break through tissues is considered to depend on proteolytic enzymes which decompose an extracellular matrix.

Page 8, please amend the first paragraph as follows:

As enzymes which decompose the matrix, mainly, metal proteases (Rha, S. Y. et al., Breast Cancer Research Treatment, 43, 175, 1997) and serine proteases are known. They cooperate to decompose matrix ~~protein~~ proteins such as collagen, laminin and fibronectin. Among the serine proteases known to be concerned in decomposition of the matrix, in particular, there is urokinase type plasminogen activator (U-PA). U-PA has a role as a trigger specific for a protein decomposition chain reaction. Its direct target is plasminogen. It is present in blood abundantly and is a precursor of an inactive serine protease which accumulates in reconstructed sites of tissues such as injured sites and tumors as well as inflammatory sites. In addition, as proteases which are concerned in metastasis and infiltration of cancers, for example, a tissue factor, lysosomal type hydrolase and collagenase have been known.

Page 8, please amend the second paragraph as follows:

At present, cancer is the top cause of death in Japan and more than 200,000 people ~~are died~~ die per year. ~~Then~~ Accordingly, specific substances which can be used as markers for diagnosis and therapy or prophylaxis of cancer are studied intensively. Such specific substances are referred to as tumor markers or tumor marker relating biomarkers. They are utilized in aid of diagnosis before treatment of cancer, for presuming carcinogenic organ and pathological tissue type, for monitoring

effect of treatment, for finding recurrence early, for presuming prognosis, and the like. At present, tumor markers are essential in clinical analyses. Among them, alpha fetoprotein (AFP) which has high specificity to hepatocellular carcinoma and yolk sac tumor (Taketa K. et al., Tumour Biol., 9, 110, 1988), and carcinoembronic antigen (CEA) are used worldwide. In the future, tumor markers will be required more and more, and it is desired to develop, for example, organ specific markers and tumor cell specific markers which are highly reliable serologic diagnosis of cancer. Up to now, humunglandular kallikrein (hK2) which is a serine protease expressed at human prostatic epithelial cells has been reported as a marker for prostatic cancer. And, hK2 has 78% homology with the sequence of prostatic specific antigen (PSA) and PSA is also used widely as a biochemical marker of prostatic cancer (Mikolajczyk, S. d. et al., Prostate, 34, 44, 1998; Pannek, J. et al., Oncology, 11, 1273, 1997; Chu, T. M. et al., Tumour Biology, 18, 123, 1997; Hsieh, M. et al., Cancer Res., 57, 2651, 1997). Further, hK2 is reported to be useful as a marker for not only prostatic cancer but also stomach cancer (Cho, J. Y. et al.. Cancer, 79, 878, 1997). Moreover, CYFRA (CYFRA 211) for measuring cytokeratin 19 fragment in serum is reported to be useful for lung cancer (Sugiyama, Y. et al., Japan J. Cancer Res., 85, 1178, 1994). Gastrin release peptide precursor (ProGRP) is reported to be useful as a tumor marker (Yamaguchi, K. et al., Japan, J. Cancer Res., 86, 698, 1995).

Page 10, please amend the second paragraph as follows:

Under these circumstances, the present inventors have succeeded in cloning ~~of~~ cDNA encoding novel human and mouse serine proteases.

Page 10, please amend the third paragraph as follows:

In summary, the 1st feature of the present invention is the amino acid sequences of ~~biological~~ biologically active mature serine proteases BSSP2 and nucleotide sequences encoding the amino acid sequences.

Page 18, please amend the second paragraph as follows:

In case of northern blotting analysis, mBSSP2 shows the expression in the head of a 15-20 days mouse fetus, and in the lung, prostate and testicle of a 3 month-old mouse. hBSSP2 shows the expression in brain, skeletal muscle and liver (see Figs. 1, 2 and 5). In case of RT-PCR analysis, mBSSP2 shows the expression in the brain and testicle of a 12 day-old mouse, and hBSSP2 shows the expression in the brain and skeletal muscle. Then, the novel proteases of the present invention are presumed to play various roles in the brain, prostate, lung, testicle, skeletal muscle and liver. For example, in the brain, there is a possibility that they can be used for treatment and diagnosis of brain diseases such as Alzheimer's disease (AD), epilepsy, brain tumor and the like. Further, in other tissues, there is a possibility that BSSP2 of the present invention and a gene

encoding it can be used for treatment and diagnosis of various diseases such as cancer, inflammation, infertility, prostatomegaly and the like. Further, it is presumed they may have a certain influence on blood coagulation, fibrinolysis and complement systems. Furthermore, there is a possibility that inhibitors of serine proteases can be used for treatment and diagnosis of Alzheimer's disease, epilepsy, cancer, inflammation, infertility, prostatomegaly and the like.

Page 22, please amend the paragraph on page 22 as follows:

In general, many genes of ~~eucaryote~~ eucaryotes exhibit polymorphism and, sometimes, one or more amino acids are substituted by this phenomenon. Further, even in such a case, sometimes, a protein maintains its activity. Then, the present invention includes a gene encoding a protein obtained by modifying a gene encoding any one of the amino acid sequences represented by SEQ ID NOS: 2, 4, 6, 8 and 10, artificially, in so far as the protein has the characteristic function of the gene of the present invention. Further, the present invention includes a protein which is a modification of any one of amino acid sequences represented by SEQ ID NOS: 2, 4, 6, 8 and 10 in so far as the protein has the characteristics of the present invention. Modification is understood to include substitution, deletion, addition and/or insertion. In particular, the present inventors have shown that, even when several amino acids are

added to or deleted from the N-terminus amino acid of the BSSP2 mature protein
represented by SEQ ID NO: 2, the resultant sequence maintains its activity.

Page 23, please amend the first paragraph as follows:

That is, the present invention includes a protein comprising any one of the amino acid sequences described in SEQ ID NOS: 2, 4, 6, 8 and 10; an amino acid sequence encoded by any one of the nucleotide sequences represented by SEQ ID NOS: 1, 3, 5, 7 and 9; or one of these amino acid sequences wherein one to several amino acids have been substituted, deleted, added and/or inserted, and ~~being~~ belonging to serine protease family.

Page 23, please amend the second paragraph as follows:

Each codon for the desired amino acid itself has been known and ~~it~~ can be selected freely. For example, codons can be determined according to a conventional manner by taking into consideration ~~of~~ the frequency of use of codons in a host to be utilized (Grantham, R. et al., Nucleic Acids Res., 9, r43, 1989). Therefore, the present invention also

includes a nucleotide sequence appropriately modified by taking into consideration ~~of~~ the degeneracy of a codon. Further, these nucleotide sequences can be modified by a site directed mutagenesis using a primer composed of a synthetic oligonucleotide encoding the desired modification (Mark, D. F. et al., Proc. Natl. Acad. Sci. USA., 81, 5662, 1984), or the like.

Page 23, please amend the third paragraph as follows:

Furthermore, the DNA of the present invention includes DNA which is hybridizable to any one of the nucleotide sequences described in SEQ ID NOS: 1, 3, 5, 7 and 9 or nucleotide sequences complementary to these nucleotide sequences in so far as the protein encoded by the nucleotide sequence has the same properties as those of the BSSP2 of the present invention. It is considered that many of the sequences which are hybridizable to a given sequence under stringent conditions have a similar activity to that of a protein encoded by the given sequence. The stringent conditions according to the present invention includes, for example, incubation in a solution containing 5 x SSC, 5% Denhardt's solution (0.1% BSA, 0.1% Ficol 1400, 0.1% PVP), 0.5% SDS and 20 µg/ml denatured salmon sperm DNA at 37°C overnight, followed by washing with 2 x SSC containing 0.1% SDS at room temperature. Instead of SSC, SSPE can be appropriately used.

Page 25, please amend the second paragraph as follows:

The present invention also relates to a vector comprising the nucleotide sequence represented by SEQ ID NO: 1 or a nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 2; the nucleotide sequence represented by SEQ ID NO: 3 or a nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 4; the nucleotide sequence represented by SEQ ID NO: 5 or a nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 6; the nucleotide sequence represented by SEQ ID NO: 7 or a nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 8; or the nucleotide sequence represented by SEQ ID NO: 9 or a nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 10; or a nucleotide sequence similar to them. A nucleotide sequence similar to a ~~give~~ given nucleotide sequence used herein means a nucleotide sequence which is hybridizable to the given nucleotide sequence or its complementary nucleotide sequence under the above-described stringent conditions and which encodes a protein having the same properties as those of the protein encoded by the nucleotide sequence.

Page 28, please amend the first paragraph as follows:

The animal cells and insect cells used herein include cells derived from human ~~being~~ beings and cells derived from ~~fly~~ flies or silk ~~worm~~ worms. For example there are CHO ~~cell~~ cells, COS ~~cell~~ cells, BHK ~~cell~~ cells, Vero ~~cell~~ cells, myeloma ~~cell~~ cells, HEK293 cells, HeLa ~~cell~~ cells, Jurkat ~~cell~~ cells, mouse L ~~cell~~ cells, mouse C127 ~~cell~~ cells, 10 mouse FM3A ~~cell~~ cells, mouse fibroblast, osteablast, cartilage ~~cell~~ cells, S2, Sf9, Sf21, High Five™ (registered trade mark) ~~cell~~ cells and the like.

Page 28, please amend the second paragraph as follows:

The protein of the present invention as such can be expressed as a recombinant fused protein so as to facilitate isolation, purification and recognition. The recombinant fused protein used herein means a protein expressed as an adduct wherein a suitable peptide chain ~~are~~ is added to the N-terminus and/or C-terminus of the desired protein expressed by a nucleotide sequence encoding the desired protein. The recombinant protein used herein means that obtained by integrating a nucleotide sequence encoding the desired protein in the expression vector of the present invention and cut off an amino acid sequence which derived from nucleic acids other than those encoding the desired protein from the expressed recombinant fused protein, and is substantially the same as the protein of

the present invention.

Page 29, please amend the first paragraph as follows:

Introduction of the above vector into host cells can be carried out by, for example, transfection according to the lipopolyamine method, DEAE-dextran method, Hanahan method, lipofectin method or calcium phosphate method, microinjection, eletroporation and the like.

Page 33, please amend the first paragraph as follows:

For obtaining fertilized egg cells efficiently, ovulation may be induced with gonadotropin or the like. Fertilized egg cells are recovered and a gene in an injection pipette is injected into male pronucleus of the egg cells by microinjection. For returning the injected egg cells to a fallopian tube, an animal (false pregnancy female mouse, etc.) is provided and about 10 to 15 ~~eggs/mouse~~ eggs/mice are transplanted. Then, genomic DNA is extracted from the end part of the tail to confirm whether the transgene is introduced into newborn mouse or not. This confirmation can be carried out by detection of the transgene with southern blot technique or PCR technique, or by positive cloning wherein a marker gene, which is activated only when homologous recombination is caused, has been introduced. Further, transcribed products derived from the transgene are detected by

northern blot technique or RT-PCR technique to confirm expression of the transgene. Or, western blotting can be carried out with a specific antibody to a protein.

Page 33, please amend the second paragraph as follows:

The knockout mouse of the present invention is treated so that the function of mBSSP2 gene is lost. A knockout mouse means a transgenic mouse in which any one of ~~whose gene~~ its genes is destroyed by homologous recombination technique so that its function is deficient. A knockout mouse can be created by carrying out homologous recombination with ES cells and selecting embryonic stem cells wherein either of allele genes are modified or destroyed. For example, embryonic stem cells whose genes are manipulated at ~~blstocyte~~ the blastocyte or morula stage of fertilized eggs are injected to obtain a ~~chimera~~ chimeric mouse wherein cells derived from the embryonic stem cells are mixed with those derived from the embryo. The ~~chimera~~ chimeric mouse (~~chimera~~ chimeric means a single individual formed by somatic cells based on two or more fertilized eggs) can be mated with a normal mouse to create a heterozygote mouse wherein all of the allele genes have been modified or destroyed. Further, a homozygote mouse can be created by mating heterozygote mice.

Page 37, please amend the paragraph on page 37 as follows:

Examples of myeloma cells include X-63Ag8, NS-1, P3U1, SP2/0, AP-1 and the like with SP2/0 being preferred. The preferred ratio of the number of the antibody producer cells (spleen cells) : the number of spleen cells are 1 : 20 to 20 : 1. PEG (preferably PEG 1000 to PEG 6000) is added at a concentration of about 10 to 80% and the mixture is incubated at 20 to 40°C, preferably 30 to 37°C for 1 to 10 minutes to carry out the cell fusion efficiently. Screening of anti-hBSSP2 or mBSSP2 antibody producer hybridomas can be carried out by various methods. For example, a supernatant of a hybridoma culture is added to a solid phase to which hBSSP2 or mBSSP2 antigen is adsorbed directly or together with a carrier (e.g., microplate), followed by addition of an anti-immunoglobulin antibody (in case that the cells used in cell fusion ~~is~~ are those of a mouse, anti-mouse immunoglobulin antibody is used) or protein A to detect the anti-hBSSP2 or mBSSP2 monoclonal antibody attached to the solid phase. Or, a supernatant of a hybridoma culture is added to a solid phase to which an anti-immunoglobulin antibody or protein A is adsorbed, followed by addition of hBSSP2 or mBSSP2 labeled with a radioactive substance, an enzyme, etc., to detect the anti-hBSSP2 or mBSSP2 monoclonal antibody attached to the solid phase.

Page 38, please amend the first paragraph as follows:

Selection and cloning of the anti-hBSSP or mBSSP

monoclonal antibody can be carried out according to a per se known method or its modification. Normally, a HAT (hypoxanthine, aminopterin, thymidine)-added medium for culturing animal cells is used. Any culture medium can be used for selection, cloning and growing up in so far as the hybridoma can grow. For example, there can be used RPMI culture medium containing 1 to 20%, preferably 10 to 20% fetal bovine serum, a serum-free medium for culturing hybridomas. Preferably, the culture is carried out at a temperature of about 37°C. Normally, the culture time is 5 days to 3 weeks, preferably 1 ~~weeks~~ week to 2 weeks. Normally, the culture is carried out under 5% CO_2. The antibody titer of a supernatant of a hybridoma culture can be measured according to the same manner as that of the above-described measurement of anti-BSSP2 antibody titer in an antiserum. That is, examples of the measurement to be used include radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), FIA (fluorescence immunoassay), plaque assay, agglutination reaction method, and the like. Among them, ELISA as shown ~~blew~~ below is preferred.

Page 39, please amend the paragraph on page 39 as follows:

Normally, cloning is carried out by a per se known method such as semi-solid agar method, limiting dilution method and the like. Specifically, after confirming a well in which the desired antibody is produced by the above-described method,

cloning is carried out to obtain a single clone. For cloning, it is preferred to employ limiting dilution method wherein hybridoma cells are diluted so that one colony is formed per one well of a culture plate. For cloning by limiting dilution method, feeder cells can be used, or a cell growth factor such as interleukin 6, etc. can be added to improve colony forming capability. In addition, cloning can be carried out by using FACS and single cell manipulation method. The cloned hybridoma is preferably cultured in a serum-free culture medium and an optimal amount of an antibody is added to its supernatant. The single hybridoma thus obtained can be cultured in a large ~~about~~ amount by using a flask or a cell culture device, or cultured in the abdominal cavity of an animal (J. Immunol. Meth., 53, 313, 1982) to obtain a monoclonal antibody. When culturing in a flask, there can be used a cell culture medium (e.g., IMDM, DMEM, RPMI1640, etc.) containing 0 to 20% of FCS. When culturing in the abdominal cavity of an animal, the animal to be used is preferably the same species or the same line as that from which the myeloma cells used in the cell fusion are derived, a thymus deficient nude mouse or the like, and the hybridoma is transplanted after administration of a mineral oil such as pristane, etc. After 1 to 2 weeks, myeloma cells are proliferated in the abdominal cavity to obtain ascites containing a monoclonal antibody.

Page 42, please amend the paragraph on page 42 as follows:

The polyclonal antibody of the present invention can be produced according to a per se known method or its modification. For example, an immunogen (protein antigen) per se or a complex thereof with a carrier protein is prepared and, according to the same manner as that in the above monoclonal antibody production, a warm-blooded animal is immunized. A material containing an antibody against the protein of the present invention or its fragment is collected from the immunized animal and the antibody is separated and purified to obtain the desired antibody. As for a complex of an immunogen and a carrier protein for immunizing a warm-blooded animal, the kind of a carrier protein and the mixing ratio of a carrier and a hapten are not specifically limited in so far as an antibody against hapten immunized by cross-linking with the carrier is efficiently produced. For example, there can be used about 0.1 to 20, preferably about 1 to 5 parts by weight of bovine serum albumin, bovine cycloglobulin, hemocyanin, etc. coupled with one part by weight of a hapten. For coupling a carrier and a hapten, various condensing agents can be used. Examples thereof include glutaraldehyde, carbodiimide or maleimide active ester, active ester agents having thiol group or dithiopyridyl group, and the like. The condensed product is administered as such or together with a carrier or diluent to a site of a warm-blooded animal

where an antibody can be produced. For enhancing the antibody production, upon administration, Freund's complete adjuvant or Freund's incomplete adjuvant may be ~~administrated~~ administered. Normally, the administration is carried out once every 2 to 6 weeks, 3 to 10 times in all. The polyclonal antibody can be collected from blood, ascites, or the like, preferably blood of the immunized animal. The polyclonal antibody titer in an antiserum can be measured according to the same manner as measurement of the above monoclonal antibody titer in the antiserum. Separation and purification of the polyclonal antibody, like the above monoclonal antibody, can be carried out according to the same manner as those of immunoglobulins.

Page 44, please amend the second paragraph as follows:

As a sandwich method for determining hBSSP2 or mBSSP2 or a fragment thereof, there can be used a two step method, a one step method and the like. In ~~the~~ two step method, first, the immobilized antibody is reacted with hBSSP2 or mBSSP2 or a fragment thereof and then unreacted materials are completely removed by washing, followed by addition of the labeled antibody to form immobilized antibody-hBSSP2 or mBSSP2-labeled antibody. In ~~the~~ one step method, the immobilized antibody, labeled antibody and hBSSP2 or mBSSP2 or a fragment thereof are added at the same time.

Page 45, please amend the second paragraph as follows:

For immobilization of the antibody, a known chemical bonding method or a physical adsorption can be used. Examples of a chemical bonding method include a method using glutaraldehyde; maleimide method using ~~N-succusinimidyl-4~~ N-succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate, ~~N-succusinimidyl-2-maleimide~~ N-succinimdyl-2-maleimide acetate or the like; carbodiimide method using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride; or the like. In addition, there are maleimidobenzoyl-N-hydroxysuccinimide ester method, N-succinimidyl-3-(2-pyridylthio)propionic acid method, bisdiazobenzidine method, and dipalmityllysine method. Or, it is possible to capture a complex formed beforehand by reacting a ~~materiel~~ material to be tested with two antibodies, whose epitopes are different, with an immobilized a 3rd antibody against the antibody.

Page 46, please amend the paragraph on page 46 as follows:

For labeling, it is preferred to use an enzyme, fluorescent substance, luminous substance, radioactive substance, metal chelate, or the like. Examples of the enzyme include peroxidase, alkaline phosphatase, β-D-galactosidase, malate dehydrogenase, *Staphylococcus* nuclease, δ-5-steroidisomerase, α-glycerol phosphate dehydrogenase, triose phosphate isomerase,

horseradish peroxidase, asparaginase, glucose oxidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase, acetylcholinesterase and the like. Examples of the fluorescent substance include fluorescein isothiocyanate, phycobiliprotein, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, o-phthalaldehyde, and the like. Examples of the luminous substance include isoluminol, lucigenin, luminol, aromatic acridinium ester, imidazole, acrdinium salt and its modified ester, luciferin, luciferase, aequorin and the like. Examples of the radioactive substance include ^{125}I, ^{127}I, ^{131}I, ^{14}C, ^{3}H, ^{32}P, ^{35}S and the like. The labeling material is not limited to them and any material which can be used for immunological determination can be used. Further, a low molecular weight hapten such as biotin, dinitrophenyl, pyridoxal or fluorescamine may be attached to the antibody. Preferably, horseradish peroxidase is used as a labeling enzyme. This enzyme can be reacted with various substrates and can readily be attached to the antibody by periodate method.

Page 47, please amend the first paragraph as follows:

When an enzyme is used as a labeling material, a substrate and, if necessary, a coloring enzyme is used for measuring its activity. In case of using peroxidase as the enzyme, H_2O_2 is used as a substrate and, as a coloring agent, there can be used 2,2'-azino-di-[3-ethylbenzthiazoline sulfonic

acid] ammonium salt (ABTS), 5'-aminosalicylic acid, o-phenylenediamine, 4-aminoantipyrine, 3,3',5,5'-tetramethylbenzidine and the like. In case of using alkaline phosphatase as the enzyme, ~~o-nitorphenylphosphate~~ o-nitrophenylphosphate, p-nitrophenylphosphoric acid, or the like can be used as a substrate. In case of using β-D-galactosidase as the enzyme, fluorescein-d-(β-D-galactopyranoside), 4-methylumbelliphenyl-β-D-galactopyranoside, or the like can be used as a substrate. The present invention also ~~include~~ includes a kit comprising the above monoclonal antibody, polyclonal antibody and reagents.

Page 47, please amend the second paragraph as follows:

As a cross-linking agent, a known cross-linking agent such as N,N'-o-phenylenedimaleimide, 4-(N-maleimidomethyl)cyclohexanoate-N-succinimide ester, 6-maleimidohexanoate-N-succineimide ester, 4,4'-dithiopyridine or the like can be utilized. The reaction of these cross-linking agents with enzymes and antibodies can be carried out by a known method according to properties of a particular cross-linking agent. Further, as the antibody, a fragment thereof, for example, Fab', Fab, F(b'2) can be used as the case may be. A labeled enzyme can be obtained by the same treatment regardless of whether the antibody is polyclonal or monoclonal. When the above labeled enzyme obtained by using a cross-linking agent is

purified by a known method such as affinity chromatography or the like, ~~a~~ an immunoassay system having more higher sensitivity can be obtained. The enzyme labeled and purified antibody is stored ~~at~~ in a dark cold place with addition of a stabilizer such as thimerosal, glycerin or after lyophilization.

Page 49, please amend the paragraph on page 49 as follows:

The cloning was carried out by PCR using a mouse brain cDNA library (Clontech) as a template and nucleotide sequences corresponding to an amino acid sequence common to serine proteases represented by

Primer 1: GTG CTC ACN GCN GCB CAY TG (SEQ ID NO: 20)

Primer 2: CCV CTR WSD CCN CCN GGC GA (SEQ ID NO: 21)

as primers. Namely, 5 µl of the template, 5 µl of 10 x ExTaq buffer, 5 µl of dNTP, 10 pmol of each of the above primers and 0.5 µl of ExTaq (TAKARA) were added and the total volume was adjusted to 50 µl with sterilized water. PCR was carried out by repeating a cycle of heating at 94°C for 0.5 minute, at 55°C for 0.5 minute and then at 72°C for 1 ~~minutes~~ minute, 30 times. The PCR product was mixed with pCR II-TOPO vector attached to TOPO TA cloning kit (Invitrogen) and the mixture was allowed to stand at room temperature for 5 minutes. Then, according to a conventional manner, *E. coli* Top 10 attached to the kit was transformed and applied to a LB (Amp^+) plate (containing 100

µg/ml of ampicillin). According to a conventional manner, a plasmid was extracted from each colony obtained and its nucleotide sequence was determined by cycle sequencing method with a fluorescence sequencer (ABI). Homology of the sequence of each clone was examined by means of GenBank. Regarding an unknown sequence, i.e., BSSP2 gene, the full length cDNA was obtained by 5' RACE and 3' RACE and, according to the same manner as described above, the nucleotide sequence was determined. Namely, BSSP2 clone specific primers, GSP1 primers [mBSSP2.2 (SEQ ID NO: 27) or mBSSP2.0 (SEQ ID NO: 22)] and GSP2 primers [mBSSP2R2 (SEQ ID NO: 28) or mBSSP2.1 (SEQ ID NO: 23)] were prepared. PCR was carried out by using mouse brain Marathon-Ready cDNA (Clontech), AP1 primer attached to this reagent and either of the above GSP1 primers and heating at 94°C for 2 minutes once and repeating a cycle of heating at 94°C for 30 seconds, at 60°C for 30 seconds and then at 72°C for 30 seconds 35 times. Then, 5 µl of the PCR product diluted to 1/100, 5 µl of 10 x buffer, 5 µl of dNTP, 10 pmol of either of 10 µM of the above GSP2 primer, 10 pmol of AP2 primer attached to the above reagent and 0.5 unit of ExTaq were admixed and adjusted to 50 µl with sterilized water. Then, according to the same manner as the above, PCR was carried out. The PCR product was cloned by the above TOPO TA cloning kit and sequenced to obtain the upstream and downstream regions of the above clone. At this time, as for a clone which seemed not to cover the full length of a protein,

the specific primers shown hereinafter were prepared based on the newly found nucleotide sequence. Further, based on this sequence, the primers capable of amplifying ORF as shown hereinafter [mBSSPF7 (SEQ ID NO: 26), mBSSP2R/E (SEQ ID NO: 29)] were prepared and PCR carried out using mouse brain Marathon-ready cDNA as a template to confirm that these clones were identical. This was cloned into pCR II-TOPO vector attached to TOPO TA cloning kit to obtain the plasmid pCR II/mBSSP2 containing the full length cDNA clone. The nucleotide sequence of DNA contained in this plasmid is shown in SEQ ID NO: 7 and the amino acid sequence of mSSP2 protein deduced from the nucleotide sequence is shown in SEQ ID NO: 8. Further, two different types of clones were obtained. The nucleotide sequences of these DNA are shown in SEQ ID NOS: 3 and 5, respectively. The amino acid sequences of mBSSP2 proteins deduced from these nucleotide sequences are shown in SEQ ID NOS: 4 and 6. These novel proteases are divided into types 1, 2 and 3. Type 1 is composed of 273 amino acids, type 2 is composed of 311 amino acids and type 3 is composed of 445 amino acids. These amino acid sequences contained the common amino acid sequence composed of 238 amino acids whose N-terminus side started with Ile-Val-Gly-Gly-Gln-Ala-Val as the mature serine protease. Further, in the amino acid sequence of the mature serine protease, a consensus sequence having a serine protease activity was contained. Furthermore, since there were two or more amino acid sequence

sequences specific for a sugar chain bonding site, it was presumed that the amino acid sequence had at least two sugar chains.

Page 53, please amend the paragraph on page 53 as follows:

As seen ~~form~~ from Figs. 1 and 2, in the case of northern blotting analysis, the expression of mBSSP2 was recognized in the head of 15th to 20th day fetuses of mice and, as to the 3-month-old mice, the expression was recognized in the prostate and testicle. Further, according to the results of RT-PCR, the expression was recognized in the head of 12-day-old mice and the testicle of 3-month-old mice.

IN THE CLAIMS

20. (Amended) A vector comprising the nucleotide sequence according to ~~any one of claims 2, 4, 6, 8, 10, 12 and 14-19~~ claim 42.

21. (Amended) Transformed cells having the nucleotide sequence according to ~~any one of claims 2, 4, 6, 8, 10, 12 and 14-19~~ claim 42 in an expressible state.

22. (Amended) A process for producing a protein which

comprises culturing cells transformed with the nucleotide sequence ~~according~~(aa) to ~~any one~~ (ll), (vv) to (zz), (aaa), (bbb) or (ccc) of ~~claims 2, 4, 6, 8, 15-18~~ claim 42, and collecting mBSSP2 produced.

23. (Amended) A process for producing a protein which comprises culturing cells transformed with the nucleotide sequence ~~according~~(mm) to ~~any one~~(uu), (ddd) or (eee) of ~~claims 10, 12, 14 or 19~~ claim 42, and collecting hBSSP2 produced.

24. (Amended) The process according to claim 22 ~~or 23~~, wherein the cells are *E. coli* cells, animal cells or insect cells.

29. (Amended) An antibody against the protein according to ~~any one of claims 1, 3, 5, 7, 9, 11 and 13~~ claim 41 or a fragment thereof.

31. (Amended) A process for producing a monoclonal antibody against the protein according to ~~any one of claims 1, 3, 5, 7, 9, 11 and 13~~ claim 41 or a fragment thereof which comprises administering the protein according to ~~any one of claims 1, 3, 5, 7, 9, 11 and 13~~ claim 41 or a fragment thereof to a warm-blooded animal other than a human being, selecting the

animal whose antibody titer is recognized, collecting its spleen or lymph node, fusing the antibody producing cells contained therein with myeloma cells to prepare a monoclonal antibody producing hybridoma.

32. (Amended) A method for determining the protein according to ~~any one of claims 1, 3, 5, 7, 9, 11 and 13~~ claim 41 or a fragment thereof in a specimen which is based on immunological binding of an antigen against the protein or a fragment thereof to the protein or a fragment thereof in the specimen.

33. (Amended) A method for determining hBSSP2 or a fragment thereof in a specimen which comprises reacting a monoclonal antibody or a polyclonal antibody against the protein ~~according to any one~~ (i), (j), (k), (l), (m) or (n) of ~~claims 9, 11 and 13~~ claim 41 or a modified derivative or fragment thereof and a labeled antibody with hBSSP2 or a fragment thereof in the specimen to detect a sandwich complex produced.

34. (Amended) A method for determining hBSSP2 or a fragment thereof in a specimen which comprises reacting a monoclonal antibody or a polyclonal antibody against the protein ~~according to any one of claims 9, 11 and 13 and~~ (i), (j), (k), (l), (m) or (n) of claim 41 or a modified derivative thereof or

a fragment thereof with labeled hBBSP2 and hBSSP2 or a fragment thereof in the specimen competitively to detect an amount of hBSSP2 or a fragment thereof in the specimen based on an amount of the labeled hBBSP2 reacted with the antibody.

35. (Amended) The method according to ~~any one of claims 32-34~~ claim 32, wherein the specimen is a body fluid.

36. (Amended) A diagnostic marker for diseases in tissues comprising the protein according to ~~any one of claims 1, 3, 5, 7, 9, 11 and 13~~ claim 41.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

ATTY.'S DOCKET: UEMURA 7

In re Application of: Hidetoshi UEMURA et al.	)	Art Unit:
	)	Examiner:
I.A. No.: PCT/JP99/06475	)	Washington, D.C.
Filed: 19 November 1999	)	May 21, 2001
For: NOVEL SERINE PROTEASE BSSP2)	)	

PRELIMINARY AMENDMENT

Honorable Commissioner for Patents and Trademarks
Washington, D.C. 20231

Sir:

Contemporaneous with the filing of this case and prior to calculation of the filing fee, kindly amend as follows:

IN THE SPECIFICATION

After the title please insert the following paragraph:

REFERENCE TO RELATED APPLICATIONS

--The present application is the national stage under 35 U.S.C. §371 of international application PCT/JP99/06475, filed 19 November 1999 which designated the United States, and which application was not published in the English language.--

In re of: Hidetoshi UEMURAet al. (UEMURA 7)

REMARKS

The above amendment to the specification is being made to insert reference to the PCT application of which the present case is a U.S. national stage.

Favorable consideration is earnestly solicited.

Respectfully submitted,
BROWDY AND NEIMARK, P.L.L.C.
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NOVEL SERINE PROTEASE BSSP2

FIELD OF THE INVENTION

The present invention relates to isolated polynucleotides of human and mouse serine proteases (hereinafter referred to as "hBSSP2" and "mBSSP2", respectively, and, in case no differentiation thereof from each other is needed, simply referred to as "BSSP2"), and their homologous forms, mature forms, precursors and polymorphic variants as well as a method for detecting thereof. Further, it relates to hBSSP2 and mBSSP2 proteins, compositions containing hBSSP2 and mBSSP2 polynucleotides and proteins, as well as their production and use.

BACKGROUND OF THE INVENTION

In general, proteases are biosynthesized as inactive precursors. They undergo limited hydrolysis in molecules to convert into activated type proteases. In so far as enzymes are proteases, they have an activity for hydrolyzing a peptide bond, while their action modes are varied according to kinds of proteases. According to a particular kind of catalytic site, proteases are divided into serine proteases, cysteine proteases, aspartate

proteases, metal proteases and the like. Proteases of each kind have a variety of properties, ranging from a protease having general digestive properties to a protease having various regulatory domains and strict substrate specificity, thereby specifically hydrolyzing only characteristic proteins.

Further, proteins undergo various processing even after translation to produce active proteins. In many secretory proteins, a protein are first synthesized on the ribosome in cytoplasm as an inactive precursor (pro-form) which comprises an active protein bearing at the N-terminus thereof a peptide of about 15 to 60 amino acids responsible for secretion (secretory signal). This peptide region is concerned with the mechanism for passing through the cell membrane and is removed upon cleavage by a specific protease during the passage through the membrane, in almost all the cases, to produce the mature form. A secretory signal has a broad hydrophobic region comprising hydrophobic amino acids in the middle of the sequence, and basic amino acid residues at a site close to the N-terminus. A secretory signal is a synonym of a signal peptide. In addition, in some proteins, a peptide moiety which functions as a secretory signal is further attached to the N-terminus of the inactive precursor (pro-form). Such a protein is called a prepro-protein (prepro-form).

For example, trypsin is present as a prepro-form immediately after translation into amino acids. After being secreted from cells, it is present as a pro-form and is converted into active trypsin in duodenum upon limited hydrolysis by enteropeptidase or by trypsin itself.

The optimal pH range of serine proteases is neutral to weak alkaline and, in general, many of them have a molecular weight of about 30,000 or lower. All proteases of blood coagulation, fibrinolysis and complement systems having a large molecular weight belong to trypsin-like serine proteases. They have many regulator domains and form a protease cascade which is of very importance to reactions in a living body.

Recently, cDNAs and amino acid sequences of many novel proteases have been determined by PCR for consensus sequences of serine proteases using oligonucleotide primers. According to this method, novel proteases have been found by various researchers such as Yamamura et al. (Yamanura, Y et al., Biochem. Biophys. Res. Commun., 239, 386, 1997), Gschwend, et al. (Gschwend, T. P. et al., Mol. Cell. Neurosci., 9. 207, 1997), Chen et al. (Chen, Z-L, et al., J. Neurosci., 15, 5088, 1995) and others.

SEQ ID NO: 3 of JP 9-149790 A discloses neurosin as a novel serine protease. Neurosin has also been reported in Biochimica et Byophysica Acta, 1350, 11-14,

1997. By this, there is provided a method for mass production of neurosin using the serine protease gene and a method for screening specific inhibitors using the enzyme. In addition, the screening method has been shown to be useful for screening medicines for treating various diseases.

Serine proteases expressed in a brain-nerve system such as neurosin are considered to play various roles in the brain-nerve system. Therefore, there is a possibility that isolation of a gene encoding a novel protease expressed in a brain-nerve system and production of a protein using the gene would be useful for diagnosis or therapeutic of various diseases related to the brain-nerve system.

Nowadays, in general, clinical diagnosis of Alzheimer's disease is conducted based on the diagnosis standard of DSM-IIIR and NINCDS-ADRDA (Mckhann, G. et al., Neurology, 34. 939, 1994) or the diagnosis standard of DSM-IV (American Psychiatric Association; Diagnostic and statistical manuals of mental disorders, 4th ed., Washington DC, American Psychiatric Association, 1994). However, these standards are conditioned by decline of recognition functions which causes a severe disability in a daily life or a social life. Then, it is pointed out that the diagnosis is less scientific objectivity because the

diagnosis may be influenced by the level of an individual's social life and further the specialty and experience of a physician who diagnoses particular conditions. In addition, definite diagnosis of Alzheimer's disease is conducted by pathohistological analyses and, in this respect, substantial inconsistency between clinical diagnosis and autopsy diagnosis is pointed out.

At present, image diagnosis is employed as a supplemental means in clinical diagnosis of Alzheimer's diagnosis and it is possible to analyze brain functions, for example, decline of metabolism and atrophy in specific sites such as hippocampus, parietal lobe of cerebral cortex and the like which are specific for Alzheimer's disease by PET and SPECT. However, to define Alzheimer's disease based on lowering of a blood flow from parietal lobe to temporal lobe is very dangerous. In addition, there is few report showing that MRS testicle useful for patients with dementia including those of Alzheimer's disease. Further, although CT-MRI image diagnosis is used, a lesion of white matter such as atrophy of brain, PVL or the like is not specific for Alzheimer type dementia. Since it has been reported that atrophy of brain proceeds as getting older, the above observation is not necessarily found in Alzheimer type dementia. Furthermore, since an image obtained by MRI varies according to strength of a magnetic field,

performance of an apparatus and imaging conditions, numerical data obtain in different facilities cannot be compared with each other except atrophic change. In addition, there is a limit to image measurement. Further, enlargement of ventricle can be recognized in vascular dementia cases and there are cases wherein atrophy of hippocampus is observed after ischemia of basilar artery.

Under these circumstances, many researchers have requested to develop biological diagnosis markers as a means for providing better precision and objectivity for clinical diagnosis of Alzheimer's disease. At the same time, the following important roles in the future will be expected.

1) Objective judgment system of effect of medicaments for treating Alzheimer's disease.

2) Detection of Alzheimer's disease before a diagnosis standard is met, or disease conditions are manifested.

Further, data obtained in different facilities can be compared with each other by using the same diagnosis marker. Therefore, development of biological diagnosis markers is recognized to be a most important field among fields of Alzheimer's disease studies and its future prospects will be expected. Approaches to development of biological diagnosis markers up to now are divided into

that based on constitute components of characteristic pathological changes of Alzheimer's disease such as senile plaque and neurofibril change, and an approach based on other measures. Examples of the former include cerebrospinal fluid tau protein, Aβ and its precursor, βAPP. Examples of the latter include mydriasis test with cholilytic drug, Apo E and other genes relating to Alzheimer's disease. However, no good results are obtained.

Serine proteases are also considered to play important role in cancer cells. The reason why extermination of cancer by surgical treatment or topical irradiation of radioactive ray is difficult is metastasis capability of cancer. For spread of solid tumor cells in a body, they should loosen their adhesion to original adjacent cells, followed by separating from an original tissue, passing through other tissues to reach blood vessel or lymph node, entering into the circulatory system through stratum basal and endothelial layer of the vessel, leave from the circulatory system at somewhere in the body, and surviving and proliferating in a new environment. While adhesion to adjacent epidermal cells is lost when expression of cadherin which is an intercellular adhesive molecule of epithelium is stopped, to break through tissues is considered to depend on proteolytic enzymes which decompose an extracellular matrix.

As enzymes which decompose the matrix, mainly, metal proteases (Rha, S. Y. et al., Breast Cancer Research Treatment, 43, 175, 1997) and serine proteases are known. They cooperate to decompose matrix protein such as collagen, laminin and fibronectin. Among serine proteases known to be concerned in decomposition of the matrix, in particular, there is urokinase type plasminogen activator (U-PA). U-PA has a role as a trigger specific for a protein decomposition chain reaction. Its direct target is plasminogen. It is present in blood abundantly and is a precursor of an inactive serine protease which accumulates in reconstructed sites of tissues such as injured sites and tumors as well as inflammatory sites. In addition, as proteases which are concerned in metastasis and infiltration of cancers, for example, a tissue factor, lysosomal type hydrolase and collagenase have been known.

At present, cancer is the top cause of death in Japan and more than 200,000 people are died per year. Then, specific substances which can be used as markers for diagnosis and therapy or prophylaxis of cancer are studied intensively. Such specific substances are referred to as tumor markers or tumor marker relating biomarkers. They are utilized in aid of diagnosis before treatment of cancer, for presuming carcinogenic organ and pathological tissue type, for monitoring effect of treatment, for finding

recurrence early, for presuming prognosis, and the like. At present, tumor markers are essential in clinical analyses. Among them, alpha fetoprotein (AFP) which has high specificity to hepatocellular carcinoma and yolk sac tumor (Taketa K. et al., Tumour Biol., 9, 110, 1988), and carcinoembronic antigen (CEA) are used worldwide. In the future, tumor markers will be required more and more, and it is desired to develop, for example, organ specific markers and tumor cell specific markers which are highly reliable serologic diagnosis of cancer. Up to now, humunglandular kallikrein (hK2) which is a serine protease expressed at human prostatic epithelial cells has been reported as a marker for prostatic cancer. And, hK2 has 78% homology with the sequence of prostatic specific antigen (PSA) and PSA is also used widely as a biochemical marker of prostatic cancer (Mikolajczyk, S. d. et al., Prostate, 34, 44, 1998; Pannek, J. et al., Oncology, 11, 1273, 1997; Chu, T. M. et al., Tumour Biology, 18, 123, 1997; Hsieh, M. et al., Cancer Res., 57, 2651, 1997). Further, hK2 is reported to be useful as a marker for not only prostatic cancer but also stomach cancer (Cho, J. Y. et al.. Cancer, 79, 878, 1997). Moreover, CYFRA (CYFRA 21-1) for measuring cytokeratin 19 fragment in serum is reported to be useful for lung cancer (Sugiyama, Y. et al., Japan J. Cancer Res., 85, 1178, 1994). Gastrin release

peptide precursor (ProGRP) is reported to be useful as a tumor marker (Yamaguchi, K. et al., Japan, J. Cancer Res., 86, 698, 1995).

OBJECTS OF THE INVENTION

Thus, the main object of the present invention is to provide a novel serine protease which can be used for treating or diagnosing various diseases such as Alzheimer's disease (AD), epilepsy, cancer, inflammation, infertility, prostatomegaly and the like in various tissues such as brain, lung, prostate, testicle, skeletal muscle, liver and the like, and can be used as an excellent marker instead of that presently used.

SUMMARY OF THE INVENTION

Under these circumstances, the present inventors have succeeded in cloning of cDNA encoding novel human and mouse serine proteases.

In summary, the 1st feature of the present invention is amino acid sequences of biological active mature serine proteases BSSP2 and nucleotide sequences encoding the amino acid sequences.

That is, they are the amino acid sequence composed of 238 amino acids (mature type BSSP2 (SEQ ID NO: 2)) and a nucleotide sequence encoding the amino acid

sequence (the 1st to 714th bases of SEQ ID NO: 1). In addition, they include amino acid sequences substantially similar to SEQ ID NO: 2 and nucleotide sequences encoding such similar amino acid sequences. Further, they include modified derivatives of proteins having these amino acid sequences. An amino acid sequence substantially similar to a given amino acid sequence used herein means an amino acid sequence derived from the given amino acid sequence by modification such as substitution, deletion, addition and/or insertion of one to several amino acids with maintaining the same property as that of the protein having the given amino acid sequence. The modified derivative of the proteins includes, for example, phosphate adduct, sugar chain adduct, metal adduct (e.g., calcium adduct), the protein fused to another protein such as albumin etc., dimer of the protein, and the like.

In the nucleotide sequences in the Sequence Listing hereinafter, the symbol "n" represents that any of the normal bases of a nucleic acid, i.e., adenine (a), cytosine (c), guanine (g) and thymine (t) is present at that position.

The 2nd feature of the present invention is an amino acid sequence composed of 273 amino acids [type 1 BSSP2 (SEQ ID NO: 4)] wherein 35 amino acids of -35th to -1st amino acids represented by SEQ ID NO: 4 are added to

the N-terminus side of the mature BSSP2 amino acid sequence (SEQ ID NO: 2) and a nucleotide sequence encoding the amino acid sequence (247th to 1065th bases of SEQ ID NO: 3). In addition, this feature includes amino acid sequences substantially similar to SEQ ID NO: 4 and nucleotide sequences encoding these substantially similar amino acid sequences. Further, this feature includes modified derivatives of proteins having these amino acid sequences.

The 3rd feature of the present invention is an amino acid sequence composed of 311 amino acids [type 2 BSSP2 (SEQ ID NO: 6)] wherein 73 amino acids of -73rd to -1st amino acids represented by SEQ ID NO: 6 are added to the N-terminus side of the mature BSSP2 amino acid sequence (SEQ ID NO: 2) and a nucleotide sequence encoding the amino acid sequence (516th to 1448th bases of SEQ ID NO: 5). In addition, this feature includes amino acid sequences substantially similar to SEQ ID NO: 6 and nucleotide sequences encoding these substantially similar amino acid sequences. Further, this feature includes modified derivatives of proteins having there amino acid sequences.

The 4th feature of the present invention is an amino acid sequence composed of 445 amino acids [type 3 BSSP2 (SEQ ID NO: 8)] wherein 207 amino acids of -207th to -1st amino acids represented by SEQ ID NO: 8 are added to the N-terminus side of the mature BSSP2 amino acid sequence

(SEQ ID NO: 2) and a nucleotide sequence encoding the amino acid sequence (116th to 1450th bases of SEQ ID NO: 7). In addition, this feature includes amino acid sequences substantially similar to SEQ ID NO: 8 and nucleotide sequences encoding these substantially similar amino acid sequences. Further, this feature includes modified derivatives of proteins having there amino acid sequences.

The 5th feature of the present invention is an amino acid sequence of a biologically active, mature human serine protease, hBSSP2, and a nucleotide sequence encoding the amino acid sequence. That is, they are an amino acid sequence [mature type hBSSP2 (SEQ ID NO: 10) composed of 240 amino acids represented by SEQ ID NO: 10 (1st to 240th amino acids) and a nucleotide sequence encoding the amino acid sequence (807th to 1526th bases of SEQ ID NO: 9). In addition, this feature includes amino acid sequences substantially similar to SEQ ID NO: 10 (1st to 240th amino acids) and nucleotide sequences encoding these substantially similar amino acid sequences. Further, this feature includes modified derivatives of proteins having there amino acid sequences.

The 6th feature of the present invention is an amino acid sequence composed of 457 amino acids (-217th to 240th amino acids of SEQ ID NO: 10) wherein 217 amino acids of -217th to -1st amino acids represented by SEQ ID NO: 10

are added to the N-terminus side of the mature human serine protease hBSSP2 amino acid sequence (1st to 240 amino acids of SEQ ID NO: 10) and a nucleotide sequence encoding the amino acid sequence (156th to 1526th bases of SEQ ID NO: 9). In addition, this feature includes amino acid sequences substantially similar to SEQ ID NO: 10 and nucleotide sequences encoding these substantially similar amino acid sequences. Further, this feature includes modified derivatives of proteins having there amino acid sequences.

The 7th feature of the present invention is an amino acid sequence composed of 217 amino acids of -217th to -1st amino acids of SEQ ID NO: 10 and a nucleotide sequence encoding the amino acid sequence (156th to 806th bases of SEQ ID NO: 9). In addition, this feature includes amino acid sequences substantially similar to the amino acid composed of 217 amino acids of -217th to -1st SEQ ID NO: 10 and nucleotide sequences encoding these substantially similar amino acid sequences. Further, this feature includes modified derivatives of proteins having there amino acid sequences.

The present invention also relates to the nucleotide sequences represented by SEQ ID NOS: 1, 3, 5, 7 and 9 as well as nucleotide sequences similar to them.

The 8th feature of the present invention is a vector comprising the nucleotide sequence according to any

of the above 1st to the 7th feature, and transformant cells transformed with the vector.

The 9th feature of the present invention is a process for producing BSSP2 protein from the transformed cells of the 8th feature.

The 10th feature of the present invention is a transgenic non-human animal, wherein the expression level of BSSP2 gene has been altered.

The 11th feature of the present invention is an antibody against BSSP2 protein or its fragment and a process for producing thereof.

The 12th feature of the present invention is a method for determining BSSP2 protein or its fragment in a specimen using the antibody of the 11th feature.

The 13th feature is a diagnostic marker of diseases comprising BSSP2 protein.

Hereinafter, unless otherwise stated, the nucleotide sequence represented by each SEQ ID NO: includes the above-described various fragments thereof, and similar nucleotide sequences and their fragments. Likewise, the amino acid sequence represented by each SEQ ID NO: includes the above-described various fragments thereof, similar nucleotide sequences and their fragments, and modified derivatives thereof. In addition, unless otherwise stated, BSSP2, hBSSP2, and mBSSP2 include proteins having the

above-described respective amino acid sequences.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 illustrates the results of northern blotting using mRNAs prepared from mice in Example 2 hereinafter.

Fig. 2 illustrates the results of northern blotting using mRNAs prepared from mice in Example 2 hereinafter.

Fig. 3 is a plasmid constructed by the method of Example 4 hereinafter.

Fig. 4 illustrates the construction of plasmid pFBTrypSigTag/BSSP2 according to the method of Example 4 hereinafter.

Fig. 5 illustrates the detection of hBSSP2 mRNA by northern hybridization.

Fig. 6 illustrates the detection of hBSSP2 mRNA by RT-PCR.

Fig. 7 illustrates the expression of hBSSP2 by a baculovirus system.

DETAILED DESCRIPTION OF THE INVENTION

The nucleotide sequences encoding hBSSP2 or mBSSP2 of the present invention can be obtained by preparing mRNAs from cells expressing the protein and

converting it into double stranded DNAs according to a conventional manner. For preparing mRNA, guanidine isothiocyanate-calcium chloride method (Chirwin, et al., Biochemistry, 18, 5294, 1979) or the like can be used. For preparing poly (A) + RNA from total RNAs, there can be used affinity chromatography using a carrier, for example, Sepharose, latex particles, etc., to which oligo (dT) is attached, and the like. The above-obtained RNA can be used as a template and treated with reverse transcriptase by using, as a primer, oligo (dT) which is complementary to the poly (A) strand at the 3'-terminus, or a random primer, or a synthesized oligonucleotide corresponding to a part of the amino acid sequence of hBSSP2 or mBSSP2 to obtain a hybrid mRNA strand comprising DNA or cDNA complementary to the mRNA. The double stranded DNA can be obtained by treating the above-obtained hybrid mRNA strand with *E. coli* RNase, *E. coli* DNA polymerase and *E. coli* DNA ligase to convert into a DNA strand.

It is also possible to carry out cloning by RT-PCR method using primers synthesized on the basis of the nucleotide sequence of hBSSP2 or mBSSP2 gene and using hBSSP2 or mBSSP2 expressing cell poly (A) + RNA as a template. Alternatively, the desired cDNA can be obtained without using PCR by preparing or synthesizing a probe on the basis of the nucleotide sequence of hBSSP2 or mBSSP2

gene and screening a cDNA library directly. Among genes obtained by these methods, the gene of the present invention can be selected by confirming a nucleotide sequence thereof. The gene of the present invention can also be prepared according to a conventional method using chemical syntheses of nucleic acids, for example, phosphoamidite method (Mattencci, M. D. et al., J. Am. Chem. Soc., 130, 3185, 1981) and the like.

By using the thus-obtained hBSSP2 or mBSSP2 gene, their expression in various tissues can be examined.

In case of northern blotting analysis, mBSSP2 shows the expression in head of 15-20 days mouse fetus, and in lung, prostate and testicle of 3 month-old mouse. hBSSP2 shows the expression in brain, skeletal muscle and liver (see Figs. 1, 2 and 5). In case of RT-PCR analysis, mBSSP2 shows the expression in brain and testicle of 12 day-old mouse, and hBSSP2 shows the expression in brain and skeletal muscle. Then, the novel proteases of the present invention are presumed to play various roles in brain, prostate, lung, testicle, skeletal muscle and liver. For example, in brain, there is a possibility that they can be used for treatment and diagnosis of brain diseases such as Alzheimer's disease (AD), epilepsy, brain tumor and the like. Further, in other tissues, there is a possibility that BSSP2 of the present invention and a gene encoding it

can be used for treatment and diagnosis of various diseases such as cancer, inflammation, infertility, prostatomegaly and the like. Further, it is presumed they may have a certain influence on blood coagulation, fibrinolysis and complement systems. Furthermore, there is a possibility that inhibitors of serine proteases can be used for treatment and diagnosis of Alzheimer's disease, epilepsy, cancer, inflammation, infertility, prostatomegaly and the like.

The novel mouse serine protease can be divided into types 1, 2 and 3. It has been shown that type 1 is composed of 273 amino acids, type 2 is composed of 311 amino acids, and type 3 is composed of 445 amino acids. These amino acid sequences contain a common amino acid sequence of 238 amino acids whose N-terminus side starts with Ile-Val-Gly-Gly-Gln-Ala-Val as the mature serine protease. Further, the amino acid sequence of the mature serine protease contains a consensus sequence having serine protease activity. Since there are two or more amino acid sequences which are characteristic of sugar chain binding sites, the amino acid sequence is presumed to have at least two sugar chains.

Furthermore, in the novel human serine protease (hBSSP2), there are a transmembrane region and a scavenger receptor cysteine rich-like domain in the N-terminus side

of hBSSP2 mature protein as seen from SEQ ID NO: 10.

The term "pro part" used herein means a part of a pro-form, i.e., the pro-form from which the corresponding active type protein part is removed. The term "pre part" used herein means a part of a prepro-form, i.e., the prepro-form from which the corresponding pro-form is removed. The term "prepro part" used herein means a part of a prepro-form, i.e., the prepro-form from which the corresponding active type protein part is removed.

The amino acid sequence represented by SEQ ID NO: 2 is the BSSP2 mature or active type protein composed of 238 amino acids, and the nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 1 is composed of 714 bases. The present inventors have shown that the serine protease activity is maintained even when one to several amino acids of the N-terminus of the mature type protein of the present invention is deleted or added, while the sequence represented by SEQ ID NO: 2 is preferred.

The amino acid sequence represented by SEQ ID NO: 4 is type 1 BSSP2 protein composed of 273 amino acids, and the nucleotide sequence encoding the amino acid sequence represented SEQ ID NO: 3 is composed of 1685 bases. The sequence of the -35th to -1st amino acids is the prepro or pro part and the amino acid sequence represented by SEQ ID NO: 4 is considered to be a precursor type of the BSSP2

protein.

The amino acid sequence represented by SEQ ID NO: 6 is type 2 BSSP 2 protein composed of 311 amino acids and the nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 5 is composed of 2068 bases. The sequence of the -73rd to -1st amino acids is the prepro or pro part and the amino acid sequence represented by SEQ ID NO: 6 is considered to be a precursor type of BSSP2 protein.

The amino acid sequence represented by SEQ ID NO: 8 is type 3 BSSP2 protein composed of 445 amino acids and the nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 7 is composed of 2070 bases. The amino acid sequence of the -207th to -1st amino acids is the prepro or pro part and the amino acid sequence represented by SEQ ID NO: 8 is considered to be a precursor type of BSSP2 protein.

SEQ ID NOS: 4, 6 and 8 contain the common amino acid sequence represented by SEQ ID NO: 2 as the mature BSSP2 protein. Further, each of amino acid sequences of -25th to 238th amino acids in SEQ ID NOS: 4, 6 and 8 is the consensus sequence.

The amino acid sequence represented by SEQ ID NO: 10 is hBSSP2 protein composed of 457 amino acids and the nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 9 is composed of 1371 bases.

Since a transmembrane region and a scavenger receptor cysteine rich-like domain are present in the amino acid sequence of the -217th to -1st amino acids of SEQ ID NO: 10, it is considered that hBSSP2 exhibits its activity not only in the form of the mature protein but also in the form of an adduct of the -217th to -1st amino acids.

In general, many genes of eucaryote exhibit polymorphism and, sometimes, one or more amino acids are substituted by this phenomenon. Further, even in such case, sometimes, a protein maintains its activity. Then, the present invention includes a gene encoding a protein obtained by modifying a gene encoding any one of the amino acid sequences represented by SEQ ID NOS: 2, 4, 6, 8 and 10, artificially, in so far as the protein has the characteristic function of the gene of the present invention. Further, the present invention includes a protein which is a modification of any one of amino acid sequences represented by SEQ ID NOS: 2, 4, 6, 8 and 10 in so far as the protein has the characteristics of the present invention. Modification is understood to include substitution, deletion, addition and/or insertion. In particular, the present inventors have shown that, even when several amino acids are added to or deleted from the N-terminus amino acid of the BSSP2 mature protein represented by SEQ ID NO: 2, the resultant sequence

maintains its activity.

That is, the present invention includes a protein comprising any one of amino acid sequences described in SEQ ID NOS: 2, 4, 6, 8 and 10; an amino acid sequence encoded by any one of nucleotide sequences represented by SEQ ID NOS: 1, 3, 5, 7 and 9; or one of these amino acid sequences wherein one to several amino acids have been substituted, deleted, added and/or inserted, and being belonging to serine protease family.

Each codon for the desired amino acid itself has been known and it can be selected freely. For example, codons can be determined according to a conventional manner by taking into consideration of frequency of use of codons in a host to be utilized (Grantham, R. et al., Nucleic Acids Res., 9, r43, 1989). Therefore, the present invention also includes a nucleotide sequence appropriately modified by taking into consideration of degeneracy of a codon. Further, these nucleotide sequences can be modified by a site directed mutagenesis using a primer composed of a synthetic oligonucleotide encoding the desired modification (Mark, D. F. et al., Proc. Natl. Acad. Sci. USA., 81, 5662, 1984), or the like.

Furthermore, the DNA of the present invention includes DNA which is hybridizable to any one of nucleotide sequences described in SEQ ID NOS: 1, 3, 5, 7 and 9 or

nucleotide sequences complementary to these nucleotide sequences in so far as the protein encoded by the nucleotide sequence has the same properties as those of the BSSP2 of the present invention. It is considered that many of sequences which are hybridizable to a given sequence under stringent conditions have a similar activity to that of a protein encoded by the given sequence. The stringent conditions according to the present invention includes, for example, incubation in a solution containing 5 x SSC, 5% Denhardt's solution (0.1% BSA, 0.1% Ficol 1400, 0.1% PVP), 0.5% SDS and 20 μg/ml denatured salmon sperm DNA at 37°C overnight, followed by washing with 2 x SSC containing 0.1% SDS at room temperature. Instead of SSC, SSPE can be appropriately used.

Probes for detecting a BSSP2 gene can be designed based on any one of nucleotide sequences described in SEQ ID NOS: 1, 3, 5, 7 and 9. Or, primers can be designed for amplifying DNA or RNA containing the nucleotide sequence. To design probes or primers is carried out routinely by a person skilled in the art. An oligonucleotide having a designed nucleotide sequence can be synthesized chemically. And, when a suitable label is added to the oligonucleotide, the resultant oligonucleotide can be utilized in various hybridization assay. Or, it can be utilized in nucleic acid synthesis reactions such as PCR. An oligonucleotide

to be utilized as a primer has, preferably, at least 10 bases, more preferably 15 to 50 bases in length. An oligonucleotide to be utilized as a probe has, preferably, 100 bases to full length.

Moreover, it is possible to obtain a promoter region and an enhancer region of a BSSP2 gene present in the genome based on the cDNA nucleotide sequence of BSSP2 provided by the present invention. Specifically, these control regions can be obtained according to the same manner as described in JP 6-181767 A; J. Immunol., 155, 2477, 1995; Proc. Natl. Acad. Sci., USA, 92, 3561, 1995 and the like. The promoter region used herein means a DNA region which is present upstream from a transcription initiation site and controls expression of a gene. The enhancer region used herein means a DNA region which is present in an intron, a 5'-non-translated region or a 3'-non-translated region and enhances expression of a gene.

The present invention also relates to a vector comprising the nucleotide sequence represented by SEQ ID NO: 1 or a nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 2; the nucleotide sequence represented by SEQ ID NO: 3 or a nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 4; the nucleotide sequence represented by SEQ ID NO: 5 or a nucleotide sequence encoding the amino acid

sequence represented by SEQ ID NO: 6; the nucleotide sequence represented by SEQ ID NO: 7 or a nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 8; or the nucleotide sequence represented by SEQ ID NO: 9 or a nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 10; or a nucleotide sequence similar to them. A nucleotide sequence similar to a give nucleotide sequence used herein means a nucleotide sequence which is hybridizable to the given nucleotide sequence or its complementary nucleotide sequence under the above-described stringent conditions and encodes a protein having the same properties as those of the protein encoded by the nucleotide sequence.

The vector is not specifically limited in so far as it can express the protein of the present invention. Examples thereof include pBAD/His, pRSETA, pcDNA2.1, pTrcHis2A, pYES2, pBlueBac4.5, pcDNA3.1 and pSecTag2 manufacture by Invitrogen, pET and pBAC manufactured by Novagen, pGEM manufactured by Promega, pBluescriptII manufactured by Stratagene, pGEX and pUC18/19 manufactured by Pharmacia, PfastBAC1 manufactured by GIBCO and the like. Preferably, a protein expression vector (described in the specification of a patent application entitled "Protein expression vector and its use" and filed by the same applicant on the same day) is used. This expression vector

is constructed by using pCRII-TOPO vector described in the Examples hereinafter, or a commercially available expression vector, for example pSecTag2A vector or pSecTag2B vector (Invitrogen) and integrating a secretory signal nucleotide sequence suitable for expression of the protein of the present invention, in the 3' downstream side thereof, a Tag nucleotide sequence, a cleavable nucleotide sequence and a cloning site, into which a nucleotide sequence encoding a target protein can be inserted, in this order. More specifically, it is preferred to use trypsin signal as the secretory signal, a nucleotide sequence encoding polyhistidine as the Tag nucleotide sequence, and a nucleotide sequence encoding an amino acid sequence which is susceptible to enzyme-specific cleavage, i.e., a nucleotide sequence encoding the amino acid sequence of Asp-Asp-Asp-Asp-Lys (said amino acid sequence is recognized by enterokinase, and the recombinant fusion protein is cleaved at the C-terminus part thereof) as the cleavable nucleotide sequence.

Furthermore, the present invention provides transformed cells having the nucleotide sequence of the present invention in an expressible state by means of the above vector. Preferably, host cells to be used for the transformed cells of the present invention are animal cells and insect cells. However, host cells include any cells

(including those of microorganisms) which can express a nucleotide sequence encoding the desired protein in the expression vector of the present invention and can secrete extracellularly.

The animal cells and insect cells used herein include cells derived from human being and cells derived from fly or silk worm. For example, there are CHO cell, COS cell, BHK cell, Vero cell, myeloma cell, HEK293 cells, HeLa cell, Jurkat cell, mouse L cell, mouse C127 cell, mouse FM3A cell, mouse fibroblast, osteoblast, cartilage cell, S2, Sf9, Sf21, High Five™ (registered trade mark) cell and the like.

The protein of the present invention as such can be expressed as a recombinant fused protein so as to facilitate isolation, purification and recognition. The recombinant fused protein used herein means a protein expressed as an adduct wherein a suitable peptide chain are added to the N-terminus and/or C-terminus of the desired protein expressed by a nucleotide sequence encoding the desired protein. The recombinant protein used herein means that obtained by integrating a nucleotide sequence encoding the desired protein in the expression vector of the present invention and cut off an amino acid sequence which derived from nucleic acids other than those encoding the desired protein from the expressed recombinant fused protein, and

is substantially the same as the protein of the present invention.

Introduction of the above vector into host cells can be carried out by, for example, transfection according to lipopolyamine method, DEAE-dextran method, Hanahan method, lipofectin method or calcium phosphate method, microinjection, eletroporation and the like.

As described above, the present invention also relates to a process for producing hBSSP2 of mBSSP2 comprising culturing cells transformed with the above nucleotide sequence of the present invention and collecting the produced hBSSP2 of mBSSP2. The culture of cells and separation and purification of the protein can be carried out by a per se known method.

The present invention also relates to an inhibitor of the novel serine protease of the present invention. Screening of the inhibitor can be carried out according to a per se known method such as comparing the enzyme activity upon bringing into contact with a candidate compound with that without contact with the candidate compound, or the like

The present invention relates to a non-human transgenic animal whose expression level of hBSSP2 or mBSSP2 gene has been altered. The hBSSP2 or mBSSP2 gene used herein includes cDNA, genomic DNA or synthetic DNA

encoding hBSSP2 or mBSSP2. In addition, expression of a gene includes any steps of transcription and translation. The non-human transgenic animal of the present invention is useful for studies of functions or expression control of hBSSP2 or mBSSP2, elucidation of mechanisms of diseases in which hBSSP2 or mBSSP2 is presumed to be involved, and development of disease model animals for screening and safety test of medicine.

In the present invention, expression of a gene can be modified artificially by mutagenizing at a part of several important sites which control normal gene expression (enhancer, promoter, intron, etc.) such as deletion, substitution, addition and/or insertion to increase or decrease an expression level of the gene in comparison with its inherent expression level. This mutagenesis can be carried out according to a known method to obtain the transgenic animal.

In a narrow sense, the transgenic animal means an animal wherein a foreign gene is artificially introduced into reproductive cells by gene recombinant techniques. In a broad sense, the transgenic animal includes an antisense transgenic animal the function of whose specific gene is inhibited by using antisense RNA, an animal whose specific gene is knocked out by using embryonic stem cells (ES cells), and an animal into which point mutation DNA is

introduced, and the transgenic animal means an animal into which a foreign gene is stably introduced into a chromosome at an initial stage of ontogeny and the genetic character can be transmitted to the progeny.

The transgenic animal used herein should be understood in a broad sense and includes any vertebrates other than a human being. The transgenic animal of the present invention is useful for studies of functions or expression control of BSSP2, elucidation of mechanisms of diseases associated with cells expressing in a human being, and development of disease model animals for screening and safety test of medicine.

As a technique for creating the transgenic animal, a gene is introduced into a nucleus in a pronucleus stage of egg cells with a micropipette directly under a phase-contrast microscope (microinjection, U.S. Patent 4,873,191). Further, there are a method using embryonic stem cell (ES cell), and the like. In addition, there are newly developed methods such as a method wherein a gene is introduced into a retroviral vector or adenoviral vector to infect egg cells, a sperm vector method wherein a gene is introduced into egg cells through sperms, and the like.

A sperm vector method is a gene recombinant technique wherein a foreign gene is incorporated into sperm cells by adhesion, electroporation, etc., followed by

fertilization of egg cells to introduce the foreign gene into the egg cells (M. Lavitranoet et al., Cell, 57, 717, 1989). Alternatively, an in vivo site specific gene recombinant technique such as that using cre/loxP recombinase system of bacteriophage P1, FLP recombinase system of *Saccharomyces cerevisiae*, etc. can be used. Furthermore, introduction of a transgene of the desired protein into a non-human animal using a retroviral vector has been reported.

For example, a method for creating a transgenic animal by microinjection can be carried out as follows.

First, a transgene primarily composed of a promoter responsible for expression control, a gene encoding a specific protein and a poly A signal is required. It is necessary to confirm expression modes and amounts between respective systems because an expression mode and amount of a specific molecule is influenced by a promoter activity, and transgenic animals differ from each other according to a particular system due to the difference in a copy number of an introduced transgene and a introduction site on a chromosome. An intron sequence which is spliced may be previously introduced before the poly A signal because it has been found that an expression amount varies due to a non-translation region and splicing. Purity of a gene to be used for introduction into fertilized egg cells

should be as high as possible. This is of importance. Animals to be used include a mouse for collecting fertilized eggs (5 to 6 week old), a male mouse for mating, a false pregnancy female mouse, a seminiferous tubule-ligated mouse, and the like.

For obtaining fertilized egg cells efficiently, ovulation may be induced with gonadotropin or the like. Fertilized egg cells are recovered and a gene in an injection pipette is injected into male pronucleus of the egg cells by microinjection. For returning the injected egg cells to a fallopian tube, an animal (false pregnancy female mouse, etc.) is provided and about 10 to 15 eggs/mouse are transplanted. Then, genomic DNA is extracted from the end part of the tail to confirm whether the transgene is introduced into newborn mouse or not. This confirmation can be carried out by detection of the transgene with southern blot technique or PCR technique, or by positive cloning wherein a marker gene, which is activated only when homologous recombination is caused, has been introduced. Further, transcribed products derived from the transgene are detected by northern blot technique or RT-PCR technique to confirm expression of the transgene. Or, western blotting can be carried out with a specific antibody to a protein.

The knockout mouse of the present invention is

treated so that the function of mBSSP2 gene is lost. A knockout mouse means a transgenic mouse any of whose gene is destroyed by homologous recombination technique so that its function is deficient. A knockout mouse can be created by carrying out homologous recombination with ES cells and selecting embryonic stem cells wherein either of allele genes are modified or destroyed. For example, embryonic stem cells whose genes are manipulated at blstocyte or morula stage of fertilized eggs are injected to obtain a chimera mouse wherein cells derived from the embryonic stem cells are mixed with those derived from the embryo. The chimera mouse (chimera means a single individual formed by somatic cells based on two or more fertilized eggs) can be mated with a normal mouse to create a heterozygote mouse wherein all of the allele genes have been modified or destroyed. Further, a homozygote mouse can be created by mating heterozygote mice.

Homologous recombination means recombination between two genes whose nucleotide sequences are the same or very similar to each other in terms of gene recombination mechanism. PCR can be employed to select homologous recombinant cells. A PCR reaction can be carried out by using a part of a gene to be inserted and a part of a region where the insertion is expected as primers to find out occurrence of homologous recombination in cells

which give an amplification product. Further, for causing homologous recombination in a gene expressed in embryonic stem cells, homologous recombinant cells can readily be selected by using a known method or its modification. For example, cells can be selected by joining a neomycin resistant gene to a gene to be introduced to impart neomycin resistance to cells after introduction.

The present invention also provide an antibody recognizing hBSSP2 or mBSSP2 or a fragment thereof. The antibody of the present invention includes an antibody against a protein having the amino acid sequence described in any of SEQ ID NOS: 2, 4, 6, 8 and 10 or its fragment. An antibody against hBSSP2 or mBSSP2 or a fragment thereof (e.g., polyclonal antibody, monoclonal antibody, peptide antibody) or an antiserum can be produced by using hBSSP2 or mBSSP2 or a fragment thereof, etc. as an antigen according to a per se known process for producing an antibody or an antiserum.

The hBSSP2 or mBSSP2 of a fragment thereof is administered to a site of a warm-blooded animal where an antibody can be produced by administration thereof as such or together with a diluent or carrier. For enhancing the antibody production, upon administration, Freund's complete adjuvant or Freund's incomplete adjuvant may be administrated. Normally, the administration is carried out

once every 1 to 6 weeks, 2 to 10 times in all. Examples of the warm-blooded to be used include monkey, rabbit, dog, guinea pig, mouse, rat, sheep, goat, chicken and the like with mouse and rat being preferred. As rats, for example, Wistar and SD rats are preferred. As mice, for example, BALB/c, C57BL/6 and ICR mice are preferred.

For producing monoclonal antibody producer cells, individuals whose antibody titer have been recognized are selected from warm-blooded animals, e.g., a mouse immunized with an antigen. Two to 5 days after the last immunization, the spleen or lymph node of the immunized animal is collected and antibody producer cells contained therein are subjected to cell fusion with myeloma cells to prepare a monoclonal antibody producer hybridoma. The antibody titer in an antiserum can be determined by, for example, reacting the antiserum with a labeled hBSSP2 or mBSSP2 as described hereinafter, followed by measurement of the activity bound to the antibody. The cell fusion can be carried out according to a known method, for example, that described by Koehler and Milstein (Nature, 256, 495, 1975) or its modifications (J. Immunol. Method, 39, 285, 1980; Eur. J. biochem, 118, 437, 1981; Nature, 285, 446, 1980). As a fusion promoting agent, there are polyethylene glycol (PEG), Sendai virus and the like. Preferably, PEG is used. Further, for improving fusion efficiency, lectin, poly-L-

lysine or DMSO can be appropriately added.

Examples of myeloma cells include X-63Ag8, NS-1, P3U1, SP2/0, AP-1 and the like with SP2/0 being preferred. The preferred ratio of the number of the antibody producer cells (spleen cells) : the number of spleen cells are 1 : 20 to 20 : 1. PEG (preferably PEG 1000 to PEG 6000) is added at a concentration of about 10 to 80% and the mixture is incubated at 20 to 40°C, preferably 30 to 37°C for 1 to 10 minutes to carry out the cell fusion efficiently. Screening of anti-hBSSP2 or mBSSP2 antibody producer hybridomas can be carried out by various methods. For example, a supernatant of a hybridoma culture is added to a solid phase to which hBSSP2 or mBSSP2 antigen is adsorbed directly or together with a carrier (e.g., microplate), followed by addition of an anti-immunoglobulin antibody (in case that the cells used in cell fusion is those of a mouse, anti-mouse immunoglobulin antibody is used) or protein A to detect the anti-hBSSP2 or mBSSP2 monoclonal antibody attached to the solid phase. Or, a supernatant of a hybridoma culture is added to a solid phase to which an anti-immunoglobulin antibody or protein A is adsorbed, followed by addition of hBSSP2 or mBSSP2 labeled with a radioactive substance, an enzyme, etc., to detect the anti-hBSSP2 or mBSSP2 monoclonal antibody attached to the solid phase.

Selection and cloning of the anti-hBSSP or mBSSP monoclonal antibody can be carried out according to a per se known method or its modification. Normally, a HAT (hypoxanthine, aminopterin, thymidine)-added medium for culturing animal cells is used. Any culture medium can be used for selection, cloning and growing up in so far as the hybridoma can grow. For example, there can be used RPMI culture medium containing 1 to 20%, preferably 10 to 20% fetal bovine serum, a serum-free medium for culturing hybridomas. Preferably, the culture is carried out at a temperature of about 37°C. Normally, the culture time is 5 days to 3 weeks, preferably 1 weeks to 2 weeks. Normally, the culture is carried out under 5% CO_2. The antibody titer of a supernatant of a hybridoma culture can be measured according to the same manner as that of the above-described measurement of anti-BSSP2 antibody titer in an antiserum. That is, examples of the measurement to be used include radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), FIA (fluorescence immunoassay), plaque assay, agglutination reaction method, and the like. Among them, ELISA as shown blew is preferred.

Screening by ELISA

A protein prepared according to the same operation as that for an immunogen is immobilized on the surface of each well of an ELISA plate. Next, BSA, MSA,

OVA, KLH, gelatin, skimmed milk, or the like is immobilized on each well to prevent non-specific adsorption. A supernatant of a hybridoma culture is added to each well and is allowed to stand for a given time so that an immunological reaction proceeds. Each well is washed with a washing solution such as PBS or the like. Preferably, a surfactant is added to this washing solution. An enzyme labeled secondary antibody is added and allowed to stand for a given time. As the enzyme to be used for the label, there can be used β-galactosidase, alkaline phosphatase, peroxidase and the like. After washing each well with the same washing solution, a substrate solution of the labeled enzyme used is added so that an enzymatic reaction proceeds. When the desired antibody is present in the supernatant of a hybridoma culture, the enzymatic reaction proceeds and the color of the substrate solution is changed.

Normally, cloning is carried out by a per se known method such as semi-solid agar method, limiting dilution method and the like. Specifically, after confirming a well in which the desired antibody is produced by the above-described method, cloning is carried out to obtain a single clone. For cloning, it is preferred to employ limiting dilution method wherein hybridoma cells are diluted so that one colony is formed per one well of a culture plate. For cloning by limiting dilution method,

feeder cells can be used, or a cell growth factor such as interleukin 6, etc. can be added to improve colony forming capability. In addition, cloning can be carried out by using FACS and single cell manipulation method. The cloned hybridoma is preferably cultured in a serum-free culture medium and an optimal amount of an antibody is added to its supernatant. The single hybridoma thus obtained can be cultured in a large about by using a flask or a cell culture device, or cultured in the abdominal cavity of an animal (J. Immunol. Meth., 53, 313, 1982) to obtain a monoclonal antibody. When culturing in a flask, there can be used a cell culture medium (e.g., IMDM, DMEM, RPMI1640, etc.) containing 0 to 20% of FCS. When culturing in the abdominal cavity of an animal, the animal to be used is preferably the same species or the same line as that from which the myeloma cells used in the cell fusion are derived, a thymus deficient nude mouse or the like, and the hybridoma is transplanted after administration of a mineral oil such as pristane, etc. After 1 to 2 weeks, myeloma cells are proliferated in the abdominal cavity to obtain ascites containing a monoclonal antibody.

The monoclonal antibody of the present invention which does not cross-react with other proteins can be obtained by selecting a monoclonal antibody which recognizes an epitope specific to hBSSP2 or mBSSP2. In

general, an epitope presented by an amino acid sequence composed of at least 3, preferably 7 to 20 successive amino acid residues in an amino acid sequence which constitutes a particular protein is said to be an inherent epitope of the protein. Then, a monoclonal antibody recognizing an epitope constituted by a peptide having an amino acid sequence composed of at least 3 successive amino acid residue selected from the amino acid residues disclosed in any of SEQ ID NOS: 2, 4, 6 and 8 can be said to be the monoclonal antibody specific for BSSP2 of the present invention. An epitope common to BSSP2 family can be selected by selecting an amino acid sequence conservative among the amino acid sequences described in SEQ ID NOS: 2, 4, 6, 8 and 10. Or, in case of a region containing an amino acid sequence specific for each sequence, a monoclonal antibody which can differentiate respective proteins can be selected.

Separation and purification of the anti-hBSSP2 or mBSSP2 monoclonal antibody, like a conventional polyclonal antibody, can be carried out according to the same manner as those of immunoglobulins. As a known purification method, there can be used a technique, for example, salting out, alcohol precipitation, isoelectric precipitation, electrophoresis, ammonium sulfate precipitation, absorption and desorption with an ion exchange material (e.g., DEAE),

ultrafiltration, gel filtration, or specific purification by collecting only an antibody with an antibody-binding solid phase or an active adsorber such as protein A or protein G, etc., and dissociating the binding to obtain the antibody. For preventing formation of aggregates during purification or decrease in the antibody titer, for example, human serum albumin is added at a concentration of 0.05 to 2%. Alternatively, amino acids such as glycine, α-alanine, etc., in particular, basic amino acids such as lysine, arginine, histidine, etc., saccharides such as glucose, mannitol, etc., or salts such as sodium chloride, etc. can be added. In case of IgM antibody, since it is very liable to be aggregated, it may be treated with β-propionilactone and acetic anhydride.

The polyclonal antibody of the present invention can be produced according to a per se known method or its modification. For example, an immunogen (protein antigen) per se or a complex thereof with a carrier protein is prepared and, according to the same manner as that in the above monoclonal antibody production, a warm-blooded animal is immunized. A material containing an antibody against the protein of the present invention or its fragment is collected from the immunized animal and the antibody is separated and purified to obtain the desired antibody. As for a complex of an immunogen and a carrier protein for

immunizing a warm-blooded animal, the kind of a carrier protein and the mixing ratio of a carrier and a hapten are not specifically limited in so far as an antibody against hapten immunized by cross-linking with the carrier is efficiently produced. For example, there can be used about 0.1 to 20, preferably about 1 to 5 parts by weight of bovine serum albumin, bovine cycloglobulin, hemocyanin, etc. coupled with one part by weight of a hapten. For coupling a carrier and a hapten, various condensing agents can be used. Examples thereof include glutaraldehyde, carbodiimide or maleimide active ester, active ester agents having thiol group or dithiopyridyl group, and the like. The condensed product is administered as such or together with a carrier or diluent to a site of a warm-blooded animal where an antibody can be produced. For enhancing the antibody production, upon administration, Freund's complete adjuvant or Freund's incomplete adjuvant may be administrated. Normally, the administration is carried out once every 2 to 6 weeks, 3 to 10 times in all. The polyclonal antibody can be collected from blood, ascites, or the like, preferably blood of the immunized animal. The polyclonal antibody titer in an antiserum can be measured according to the same manner as measurement of the above monoclonal antibody titer in the antiserum. Separation and purification of the polyclonal antibody, like the above

monoclonal antibody, can be carried out according to the same manner as those of immunoglobulins.

The monoclonal antibody and polyclonal antibody against hBSSP2 or mBSSP2 or a fragment thereof can be utilized for diagnosis and treatment of diseases associated with cells expressing hBSSP2 or mBSSP2. By using these antibodies, hBSSP2 or mBSSP2 or a fragment thereof can be determined based on their immunological binding to hBSSP2 or mBSSP2 or a fragment thereof of the present invention. Specifically, examples of a method for determining hBSSP2 or mBSSP2 or a fragment thereof in a specimen by using these antibodies include a sandwich method wherein the antibody attached to an insoluble carrier and the labeled antibody are reacted with hBSSP2 or mBSSP2 or a fragment thereof to form a sandwich complex and the sandwich complex is detected, as well as a competitive method wherein labeled hBSSP2 or mBSSP2, and hBSSP2 or mBSSP2 or a fragment thereof in the specimen are competitively reacted with the antibody and hBSSP2 or mBSSP2 or a fragment thereof in the specimen is determined based on the amount of the labeled antigen reacted with the antibody.

As a sandwich method for determining hBSSP2 or mBSSP2 or a fragment thereof, there can be used two step method, one step method and the like. In two step method, first, the immobilized antibody is reacted with hBSSP2 or

mBSSP2 or a fragment thereof and then unreacted materials are completely removed by washing, followed by addition of the labeled antibody to form immobilized antibody-hBSSP2 or mBSSP2-labeled antibody. In one step method, the immobilized antibody, labeled antibody and hBSSP2 or mBSSP2 or a fragment thereof are added at the same time.

Examples of an insoluble carrier used for the determination include synthetic resins such as polystyrene, polyethylene, polypropylene, polyvinyl chloride, polyester, polyacrylate, nylon, polyacetal, fluorine plastic, etc.; polysaccharides such as cellulose, agarose, etc.; glass; metal; and the like. An insoluble carrier may be shaped in various forms, for example, tray, sphere, fiber, rod plate, container, cell, test tube, and the like. The antibody adsorbed by a carrier is stored at a cold place in the presence of an appropriate preservative such as sodium azide or the like.

For immobilization of the antibody, a known chemical bonding method or a physical adsorption can be used. Examples of a chemical bonding method include a method using glutaraldehyde; maleimide method using N-succusinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate, N-succusinimidyl-2-maleimide acetate or the like; carbodiimide method using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride; or the like.

In addition, there are maleimidobenzoyl-N-hydroxysuccinimide ester method, N-succinimidyl-3-(2-pyridylthio)propionic acid method, bisdiazobenzidine method, and dipalmityllysine method. Or, it is possible to capture a complex formed beforehand by reacting a materiel to be tested with two antibodies, whose epitopes are different, with an immobilized a 3rd antibody against the antibody.

For labeling, it is preferred to use enzyme, fluorescent substance, luminous substance, radioactive substance, metal chelate, or the like. Examples of the enzyme include peroxidase, alkaline phosphatase, β-D-galactosidase, malate dehydrogenase, *Staphylococcus* nuclease, δ-5-steroidisomerase, α-glycerol phosphate dehydrogenase, triose phosphate isomerase, horseradish peroxidase, asparaginase, glucose oxidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase, acetylcholinesterase and the like. Examples of the fluorescent substance include fluorescein isothiocyanate, phycobiliprotein, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, o-phthalaldehyde, and the like. Examples of the luminous substance include isoluminol, lucigenin, luminol, aromatic acridinium ester, imidazole, acrdinium salt and its modified ester, luciferin, luciferase, aequorin and the like. Examples of the radioactive substance include ^{125}I, ^{127}I, ^{131}I, ^{14}C, ^{3}H, ^{32}P, ^{35}S

and the like. The labeling material is not limited to them and any material which can be used for immunological determination can be used. Further, a low molecular weight hapten such as biotin, dinitrophenyl, pyridoxal or fluorescamine may be attached to the antibody. Preferably, horseradish peroxidase is used as a labeling enzyme. This enzyme can be reacted with various substrates and can readily be attached to the antibody by periodate method.

When an enzyme is used as a labeling material, a substrate and, if necessary, a coloring enzyme is used for measuring its activity. In case of using peroxidase as the enzyme, H_2O_2 is used as a substrate and, as a coloring agent, there can be used 2,2'-azino-di-[3-ethylbenzthiazoline sulfonic acid] ammonium salt (ABTS), 5'-aminosalicylic acid, o-phenylenediamine, 4-aminoantipyrine, 3,3',5,5'-tetramethylbenzidine and the like. In case of using alkaline phosphatase as the enzyme, o-nitorphenylphosphate, p-nitrophenylphosphoric acid, or the like can be used as a substrate. In case of using β-D-galactosidase as the enzyme, fluorescein-d-(β-D-galactopyranoside), 4-methylumbelliphenyl-β-D-galactopyranoside, or the like can be used as a substrate. The present invention also include a kit comprising the above monoclonal antibody, polyclonal antibody and reagents.

As a cross-linking agent, a known cross-linking

agent such as N,N'-o-phenylenedimaleimide, 4-(N-maleimidomethyl)cyclohexanoate-N-succinimide ester, 6-maleimidohexanoate-N-succineimide ester, 4,4'-dithiopyridine or the like can be utilized. The reaction of these cross-linking agents with enzymes and antibodies can be carried out by a known method according to properties of a particular cross-linking agent. Further, as the antibody, a fragment thereof, for example, Fab', Fab, F(b'2) can be used as the case may be. A labeled enzyme can be obtained by the same treatment regardless of whether the antibody is polyclonal or monoclonal. When the above labeled enzyme obtained by using a cross-linking agent is purified by a known method such as affinity chromatography or the like, a immunoassay system having more higher sensitivity can be obtained. The enzyme labeled and purified antibody is stored at a dark cold place with addition of a stabilizer such as thimerosal, glycerin or after lyophilization.

An objective to be determined is not specifically limited in so far as it is a sample containing BSSP2 or a fragment thereof, or a sample containing a precursor of BSSP2 or a fragment thereof and includes body fluids such as plasma, serum, blood, serum, urine, tissue fluid, cerebrospinal fluid and the like.

The following Examples further illustrate the

present invention in detail but are not construed to limit the scope thereof.

Example 1

Cloning of novel serine protease mBSSP2 gene

The cloning was carried out by PCR using a mouse brain cDNA library (Clontech) as a template and nucleotide sequences corresponding to an amino acid sequence common to serine proteases represented by

Primer 1: GTG CTC ACN GCN GCB CAY TG (SEQ ID NO: 20)

Primer 2: CCV CTR WSD CCN CCN GGC GA (SEQ ID NO: 21)

as primers. Namely, 5 µl of the template, 5 µl of 10 x ExTaq buffer, 5 µl of dNTP, 10 pmol of each of the above primers and 0.5 µl of ExTaq (TAKARA) were added and the total volume was adjusted to 50 µl with sterilized water. PCR was carried out by repeating a cycle of heating at 94°C for 0.5 minute, at 55°C for 0.5 minute and then at 72°C for 1 minutes, 30 times. The PCR product was mixed with pCR II-TOPO vector attached to TOPO TA cloning kit (Invitrogen) and the mixture was allowed to stand at room temperature for 5 minutes. Then, according to a conventional manner, *E. coli* Top 10 attached to the kit was transformed and applied to a LB (Amp^+) plate (containing 100 µg/ml of ampicillin). According to a conventional manner, a plasmid was extracted from each colony obtained and its nucleotide sequence was determined by cycle sequencing method with a fluorescence

sequencer (ABI). Homology of the sequence of each clone was examined by means of GenBank. Regarding an unknown sequence, i.e., BSSP2 gene, the full length cDNA was obtained by 5' RACE and 3' RACE and, according to the same manner as described above, the nucleotide sequence was determined. Namely, BSSP2 clone specific primers, GSP1 primers [mBSSP2.2 (SEQ ID NO: 27) or mBSSP2.0 (SEQ ID NO: 22)] and GSP2 primers [mBSSP2R2 (SEQ ID NO: 28) or mBSSP2.1 (SEQ ID NO: 23)] were prepared. PCR was carried out by using mouse brain Marathon-Ready cDNA (Clontech), AP1 primer attached to this reagent and either of the above GSP1 primers and heating at 94°C for 2 minutes once and repeating a cycle of heating at 94°C for 30 seconds, at 60°C for 30 seconds and then at 72°C for 30 seconds 35 times. Then, 5 µl of the PCR product diluted to 1/100, 5 µl of 10 x buffer, 5 µl of dNTP, 10 pmol of either of 10 µM of the above GSP2 primer, 10 pmol of AP2 primer attached to the above reagent and 0.5 unit of ExTaq were admixed and adjusted to 50 µl with sterilized water. Then, according to the same manner as the above, PCR was carried out. The PCR product was cloned by the above TOPO TA cloning kit and sequenced to obtain the upstream and downstream regions of the above clone. At this time, as for a clone which seemed not to cover the full length of a protein, the specific primers shown hereinafter were prepared based on the newly

found nucleotide sequence. Further, based on this sequence, the primers capable of amplifying ORF as shown hereinafter [mBSSPF7 (SEQ ID NO: 26), mBSSP2R/E (SEQ ID NO: 29)] were prepared and PCR carried out using mouse brain Marathon-ready cDNA as a template to confirm that these clones were identical. This was cloned into pCR II-TOPO vector attached to TOPO TA cloning kit to obtain the plasmid pCR II/mBSSP2 containing the full length cDNA clone. The nucleotide sequence of DNA contained in this plasmid is shown in SEQ ID NO: 7 and the amino acid sequence of mSSP2 protein deduced from the nucleotide sequence is shown in SEQ ID NO: 8. Further, two different types of clones were obtained. The nucleotide sequences of these DNA are shown in SEQ ID NOS: 3 and 5, respectively. The amino acid sequences of mBSSP2 proteins deduced from these nucleotide sequences are shown in SEQ ID NOS: 4 and 6. These novel proteases are divided into types 1, 2 and 3. Type 1 is composed of 273 amino acids, type 2 is composed of 311 amino acids and type 3 is composed of 445 amino acids. These amino acid sequences contained the common amino acid sequence composed of 238 amino acids whose N-terminus side started with Ile-Val-Gly-Gly-Gln-Ala-Val as the mature serine protease. Further, in the amino acid sequence of the mature serine protease, a consensus sequence having a serine protease activity was contained. Furthermore, since

there were two or more amino acid sequence specific for a sugar chain bonding site, it was presumed that the amino acid sequence had at least two sugar chains.

Table 1

SEQ ID NO:	Name of primer	Direction	Sequence	Use
22	mBSSP2.0	Forward	ATGGTGGAGAAGATCATTCCT	RACE
23	mBSSP2.1	Forward	TACAGTGCCCAGAACCATG	RACE
24	mBSSPF4	Forward	CTCAACTCTCTGCTAGACCG	RACE
25	mBSSP2F5	Forward	ATAGTTGGCGGCCAAGCTGT	mature
26	mBSSPF7	Forward	CCCAGCAGAACTTACTGCCT	FL*
27	mBSSP2.2	Reverse	TGTTGCAGAGGTGGGTGCTG	RACE
28	mBSSP2R2	Reverse	TACCATTGTGTCCTGCAGTGT	RACE
29	mBSSP2R5/E	Reverse	TGAATTCTGCTGCTTCTTCGGCTAGCG	FL*

*: for full length

Example 2

Expression mBSSP2 gene in mice internal organs

According to the protocol of QuickPrep Micro mRNA purification Kit (Amersham-Pharmacia), mRNAs were isolated from various internal organs of Balb/c mice or their fetuses. They were subjected to electrophoresis according to a conventional manner and transcribed to a nylon membrane. A probe was prepared separately by isolating a part of a nucleotide sequence encoding the mature protein

of mBSSP2 from pCR II/mBSSP2, purifying it and labeling it with α-^{32}P dCTP. The probe was diluted with 5 x SSC and reacted with the above membrane filter at 65°C for a whole day and night. Then, the filter was washed twice each with 2 x SSC/0.1% SDS at room temperature for 30 minutes, 1 x SSC/0.1% SDS at room temperature for 30 minutes and 0.1 x SSC/0.1% SDS at 65°C for 30 minutes. The filter was exposed to an imaging plate for FLA2000 (Fuji Film) for one day to analyze the expression. The results shown in the drawings are those obtained by using mRNAs prepared from head of fetuses of mice and mRNAs prepared from brain of 5-day-, 10-day-, 14-day-, 18-day-, 30-day-, 3-month-, 7-month and 1-year-old mice (Fig. 1) and mRNAs prepared from various internal organs of 3-month-old mice (Fig. 2). In addition, the mRNAs of mice prepared above were subjected to RT-PCR by using Ready To Go RT-PCR Beads (Amersham-Pharmacia) and mBSSP2 gene specific primers (SEQ ID NOS: 25 and 29) according to the protocol attached to the kit.

As seen form Figs. 1 and 2, in case of northern blotting analysis, the expression of mBSSP2 was recognized in head of 15th to 20th day fetuses of mice and, as to the 3-month-old mice, the expression was recognized in prostate and testicle. Further, according to the results of RT-PCR, the expression was recognized in head of 12-day-old mice and testicle of 3-month-old mice.

Example 3

Expression of novel serine protease mature protein encoded by mBSSP2 gene

(1) Construction of expression plasmid

A cDNA region encoding the mature protein of BSSP2 protein was amplified by PCR using the plasmid pCR II/mBSSP2 as a template (the sequence of the 1st to 717th bases of SEQ ID NO: 1 was amplified by using the primers having the sequences represented by SEQ ID NOS: 25 and 29). The PCR product was ligated to pTrc-HisB (Invitrogen) which had been digested with BamHI and blunted with mung bean nuclease. *E. coli* JM109 was transformed by the resultant and colonies formed were analyzed by PCR to obtain *E. coli* containing the desired serine protease expressing plasmid pTrcHis/mBBSP2.

The resultant *E. coli* was designated E. coli pTrcHis/mBSSP2 and deposited at National Institute of Bioscience and Human-Technology (NIBH), Agency of Industrial Science & Technology of 1-1-3 Higashi, Tsukuba-shi, Ibaraki-ken, Japan on October 29, 1998 under the accession numbers of FERM P-17033.

(2) Expression of protein by *E. coli* containing expression plasmid

A single colony of *E. coli* having the expression plasmid was inoculated in 10 ml of LB (Amp^+) culture medium

and incubated at 37°C overnight. This was inoculated in 250 ml of LB (Amp^+) culture medium and incubated at 37°C. When the absorbance at 600 nm became 0.5, 250 µl of 0.1 M IPTG (isopropyl-β-D-(-)-thiogalactopyranoside) was added and the incubation was continued for additional 5 hours. The *E. coli* was centrifuged and suspended in a cell disruption buffer (10 mM phosphate buffer pH 7.5, 1 mM EDTA) and sonicated on ice to disrupt *E. coli*. This was centrifuged at 14,000 r.p.m. for 20 minutes to obtain a precipitate. The precipitate was washed twice with a cell disruption buffer containing 0.5% Triton X-100™ and washed with water to remove Triton X-100™. Then, the resultant mixture was dissolved by soaking in a denaturation buffer containing 8 M urea (8M urea, 50 mM Tris pH8.5, 20 mM 2ME) at 37°C for 1 hour. The solution was passed through TALON metal affinity resin (Clontech), washed with the denaturation buffer containing 10 mM imidazole, and then eluted with the denaturation buffer containing 100 mM imidazole to purify the solution. The purified product was dialyzed against PBS for 3 days with exchanging the buffer every other night to obtain the protein mBSSP2-His.

Example 4

Expression of novel serine protease mature protein encoded by mBSSP2 gene by using pFBTrypSigTag/BSSP2

(1) Construction of pFBTrypSigTag/BSSP2

The sequences represented by SEQ ID NOS: 11 and 12 were subjected to annealing and digested with NheI and BamHI. The resultant fragment was inserted into pSecTag2A (Invitrogen) to obtain pSecTrypHis. Twenty units of BAmHI was added to 5 μg of pSecTrypHis vector and the vector was cleaved at 37°C over 4 hours. Then, 6 units of mung bean nuclease (TAKARA) was added thereto and reacted at room temperature (25°C) for 30 minutes to blunt the terminal ends. Further, the 3'-terminus side of the cloning site was digested cleaved with 20 units of XhoI, 1 unit of bacterial alkaline phosphatase (TAKARA) was added thereto and the reaction was carried out at 65°C for 30 minutes.

According to the same manner as that described in JP 9-149790 A or Biochim. Biophys. Acta, 1350, 11, 1997, mRNA was prepared from COLO201 cells and cDNA was synthesized to obtain the plasmid pSPORT/neurosin. cDNA of an active region of neurosin was obtained from pSPORT/neurosin by PCR using primers having the sequences represented by SEQ ID NOS: 13 and 14. Ten units of XhoI was reacted with the PCR product at 37°C for 3 hours to cleave XhoI site at the 3'-side thereof. This was inserted into pSecTrypHis by TAKARA ligation kit to obtain pSecTrypHis/neursoin (Fig. 3).

Amplification was carried out by using the primers having the sequences represented by SEQ ID NOS: 15

and 16 so that the peptide of Leu-Val-His-Gly was present at the C-terminus of the part from trypsin signal to the enterokinase recognition site of pSecTrypHis/neurosin. This was inserted between NheI and HindIII sites of pSecTag2A to construct the plasmid pTrypSig.

One μg (0.1 μl) of the plasmid pSecTab2A was treated with the restriction enzymes NheI and BamHI to completely remove a region encoding the leader sequence of IgGk. One hundred pmol portions of DANs represented by SEQ ID NOS: 40 and 41 were added to the resultant solution and the mixture was heated at 70°C for 10 minutes and subjected to annealing by allowing to stand at room temperature for 30 minutes. Two μl of I solution of DNA ligation kit Ver. 2 (TAKARA) was added to 1 μl portions of His secretory signal sequence treated by NheI and BamHI and pSecTag2A and the reaction was carried out at 16°C for 30 minutes.

To the reaction mixture was add 0.1 ml of *E. coli* competent cell XL1-Blue (STRATAGENE) and reacted on ice for 30 minutes. Then, the reaction mixture was subjected to heat shock at 42°C for 60 seconds. After standing on ice for 2 minutes, 0.9 ml of SOC culture medium (Toyo Boseki K.K.) was added thereto and the mixture was shaken with a shaker at 37°C for 1 hour. The mixture was centrifuged at 5,000 r.p.m. for 1 minutes and the supernatant was discarded. The precipitated competent cells were suspended

in the liquid remained in the centrifuge tube and the suspension was applied to 2 ampicillin LB plates containing 100 μg/ml of ampicillin in the ratio of 1 : 10. The plates were incubated at 37°C for one night. Among the colonies formed, a colony into which DNA of His secretory signal was inserted was selected by PCR to obtain pTrypHis.

A sequence of about 200 bp containing His Tag region of pTrypHis was amplified by using primers having the sequence represented by SEQ ID NOS: 16 and 17 and a fragment of about 40 bp containing His Tag and enterokinase recognizing site formed by digestion of HindIII and BamHI was inserted into pTrypSig to construct pTrypSigTag (Fig. 4A).

cDNA was prepared by PCR of the sequence from trypsin signal to enterokinase recognizing site of pTrypSigTag using primers having the sequences represented by SEQ ID NOS 14 and 18 and cut out by digestion with BglII and BamHI. It was inserted into BamHI site of pFastBAC1. The insertion direction was confirmed by PCR using primers having the sequences represented by SEQ ID NOS: 14 and 19. A clone into which the cDNA was inserted in the direction toward transcription and translation was selected to obtain pFBTrypSigTag.

Twenty units of BamHI was added to 5 μg of pFBTrypSigTag vector and the vector was cleaved at 37°C

over 4 hours, followed by addition of 6 units of mung bean nuclease (TAKARA) and reaction at room temperature (25°C) for 30 minutes to blunt the terminal ends. Further, the 3'-side of the cloning site was cleaved by 20 units of EcoRI, followed by addition of 1 unit of bacterial alkaline phosphatase (TAKARA). The reaction was carried out at 65°C for 30 minutes.

cDNA of the active region of mBSSP2 was obtained by PCR according to a conventional manner using pTrcHis/mBSSP2 or pCRII/mBSSP2 prepared from *E. coli* pTrcHis/mBSSP2 (accession No. FERM P-17033). The resultant cDNA was inserted into pFBTrypSigTag to obtain pFBTrypSigTag/mBSSP2 (Fig. 4B). At this time, correct insertion of mBSSP2 was confirmed by determining the sequence.

Bacmid DNA was transformed with PFBTrypSigTag/mBSSP2 according to a protocol of Gibco BRL BAC-TO-BAC baculovirus expression system to prepare a recombinant bacmid having chimera BSSP2 fused with trypsinogen signal peptide, HisTag and enterokinase recognizing site. When this was expressed in Sf-9 cell according to a manual of BAC-TO-BAC baculovirus expression system, it was secreted in the culture supernatant from 2 days after infection of the virus.

(2) Determination of enzyme activity

The recombinant fused protein mSSP2 obtained in the culture supernatant was passed through a chelate column to purify it and, after dialysis, its enzyme activity was determined. First, the culture supernatant was applied to a chelate column (Ni-NTA-Agarose, Qiagen) with PBS buffer and eluted stepwise with a solution of imidazole (Wako Pure Chemical Industries, Ltd.) dissolved in PBS. The resultant imidazole-eluted fraction was applied to a PD-10 column (Pharmacia) to exchange to PBS buffer. Fifty µl of this sample was mixed with 10 µl of enterokinase (1 U/1 µl, Invitrogen) and the reaction was carried out at room temperature for 60 minutes. Each of various synthetic substrates (Peptide Laboratory, Boc-Gln-Ala-Arg-MCA, Boc-Phe-Ser-Arg-MCA, Bz-Arg-MCA, Boc-Val-Leu-Lys-MCA, Pyr-Gly-Arg-MCA, Pro-Phe-Arg-MCA, Boc-Val-Pro-Arg-MCA, Z-Arg-Arg-MCA, Arg-MCA, Z-Phe-Arg-MCA) was dissolved in DMSO and diluted with 1 M Tris-HCl (pH 8.0) to obtain a substrate solution. Fifty µl of 0.2 M substrate solution was added thereto and further the reaction was carried out at 37°C. After one hour, the fluorescence of AMC (7-amino-4-methylcoumalin) formed by the enzymatic reaction was measured at 380 nm of excitation wavelength and 460 nm of fluorescence wavelength to determine the activity.

As a result, the recombinant fused protein mBSSP2 has been shown to have serine protease activity.

Example 5

Cloning of hBSSP2 gene

Reverse transcription of 1 μg of mRNA of human fetus brain (Clontech) was carried out by using Superscript II (Gibco BRL) and oligo dT-Not I primer (5' GGCCACGCGTCGACTAGTA $C(T)_{17}$ 3') to obtain cDNA. By using this as a template, PCR was carried out with primes prepared from mBSSP2 nucleotide sequence and represented by SEQ ID NOS: 30 and 31 to obtain a cDNA fragment of hBSSP2. Namely, 5 μl of the template, 5 μl of 10 x ExTaq buffer (TAKARA), 5 μl of dNTPs, 10 pmol portions of the above primers and 0.5 μl of ExTaq (TAKARA) were adjusted to 50 μl with sterilized water and PCR was carried out by repeating a cycle of heating at 94°C for 0.5 minute, at 55°C for 0.5 minute and then at 72°C for 1 minute, 35 times. The PCR reactions described hereinafter were carried out according to the same manner as the above composition and conditions except the template and primers. The PCR product was mixed with pGEM-T Easy vector (Promega) and Takara Ligation Solution I (TAKARA) and the reaction was carried out at 16°C for 2 hours. Then, according to the same manner, *E. coli* JM109 was transformed and applied to a LB (Amp^+) plate. A plasmid was extracted from each colony formed according to a conventional manner and its nucleotide sequence was determined by dideoxy method. As for a clone having

homology to mBSSP2, full length cDNA was obtained by 5' RACE and 3' RACE and its sequence was determined as described above. PCR was carried out by using the above cDNA as a template and primers having the sequences represented by SEQ ID NOS: 30 and 37. 3' RACE was carried out by PCR using a 1/100 dilution of the above PCR product as a template and primers having the sequences represented by SEQ ID NOS: 32 and 37. As for 5' RACE, cDNA for RACE was prepared from human fetal brain mRNA (Clontech) by using Superscript II and SMART RACE cDNA amplification kit (Clontech). PCR of this cDNA was carried out by using a primer of 10 x Universal Primer Mix (attached to the kit) and a primer having the sequence represented by SEQ ID NO: 33. Further, PCR was carried out by using the 1/100 dilution of the latter PCR product, a template, Nested PCR Primer (attached to the kit) and a primer having the sequence represented by SEQ ID NO: 34. The finally obtained PCR product was subjected to TA cloning as described above and the nucleotide sequence was determined to obtain the upstream and downstream regions of the above clone. In addition, primers for amplifying the full length cDNA as represented by SEQ ID NOS: 35 and 36 were prepared based on the resultant nucleotide sequence and PCR was carried out by using the above synthetic cDNA as a template. This PCR product was cloned into pGEM-T Easy vector to

obtain the plasmid pGEM-TE/hBSSP2 containing the full length cDNA clone. The DNA sequence contained in this plasmid is shown in SEQ ID NO: 9 and hBSSP2 protein deduced from the nucleotide sequence is shown in SEQ ID NO: 10.

E. coli containing this plasmid was designated E. coli pGEM-TE/hBSSP2 and deposited at National Institute of Bioscience and Human-Technology (NIBH), Agency of Industrial Science & Technology of 1-1-3 Higashi, Tsukuba-shi, Ibaraki-ken, Japan on July 27, 1999 under the accession numbers of FERM P-17487.

Table 2

SEQ ID NO:	Name of primer	Direction	Sequence	Use
30	BSSP2SPF	Forward	ACTGCTGCCCACTGCATG	for part
31	BSSP2SPR	Reverse	CAGGGGTCCCCCGCTGTCTCC	for part
32	hBSSP2F11	Forward	GCTCTCAACTTCTCAGACAC	RACE
33	hBSSP2R12	Reverse	ACTCAGCTACCTTGGCGTAG	RACE
34	hBSSP2R11	Reverse	CCTGGAGCATATCCGAGCTG	RACE
35	hBSSR2F12	Forward	GCTTTACAACAGTGCTAC	WB*
36	hBSSP2R13/E	Reverse	TGGAATTCGAGGAAACAGCAGGACTCAG	WB*
37			TACTAGTCGACGCGTGGCC	

*: whole body

Example 6

Detection of hBSSP2 mRNA by northern blotting

Poly A + RNA extracted from respective tissues of human adults and fetuses were blotted on a membrane (Clontech) and the membrane was subjected to northern hybridization with a hBSSP2 probe. The probe was labeled by Takara BcaBEST random labeling kit (TAKARA) according to random priming method using a cDNA fragment which was amplified by using the full length of hBSSP2 as a template and the sequences represented by SEQ ID NOS: 34 and 35 as primers. The hybridization was carried out at 60°C overnight and the filter was finally washed with 0.1 x SSC and 0.1% SDS. The radioactivity was detected by FLA-2000 (Fuji Film). The signal corresponding to the adult brain was recognized at about 2.4 kb, the signal corresponding to the adult skeletal muscle was recognized at 7 kb and 1.3 kb and further the signal of the fetus liver was recognized at 7 kb (Fig. 5). The signal of the adult brain is considered to correspond to the exact nucleotide sequence and the others are considered to correspond to polymorphic forms resulted from the difference in splicing.

Example 7

Detection of hBSSP2 mRNA by RT-PCR

mRNAs of human tissues purchased from Clontech were subjected to RT-PCR against hBSSP2 by using Ready To Go RT-PCR Beads (Amersham-Pharmacia) according to the protocol attached to the kit. Expression of hBSSP2 was

recognized in brain and skeletal muscle (Fig. 6). No clear band was obtained in pancreas due to the combination of primers. This is considered to be non-specific amplification by a large amount of a serine protease present in pancreas.

Example 8

Expression of hBSSP2 by baculovirus system

The signal sequence of human trypsinogen 2 and (His)6 Tag and a sequence encoding the cleavage site of enterokinase were inserted into pFastBac1 (Gibco BRL) to obtain the plasmid pFBTrypSigTag. The mature form of hBSSP2 was inserted into the plasmid pFBTrypSigTag so that it was located in the flame (Fig. 4B). The mature form of hBSSP2 amplified by the sequences represented by SEQ ID NOS: 38 and 36 was cleaved by EcoRI and, according to the same manner as described with respect to mBSSP2, it was inserted into pFBTrySigTag to construct pFastBacTrypSigTag/hBSSP2. At this time, correct insertion of BSSP2 was confirmed by determining the nucleotide sequence by using the fluorescent labeled sequence represented by SEQ ID NO: 39. Bacmid DNA was transformed with PFBTrypSigTag/hBSSP2 according to a protocol of Gibco BRL BAC-TO-BAC baculovirus expression system to prepare a recombinant bacmid having chimera BSSP2 fused with trypsinogen signal peptide, HisTag and enterokinase

recognizing site. When this was expressed in Sf-9 cell according to a manual of BAC-TO-BAC baculovirus expression system and the culture supernatant from 3 days after infection of the virus subjected to western blot technique with anti-DDDDK antibody, a specific band was detected to confirm expression of hBSSP2 (Fig. 7).

Table 3

SEQ ID NO:	Name of primer	Direction	Sequence	Use
38	hBSSP2F13	Forward	ACTGCTGCCCACTGCATG	for part
39	FBTrypSigTagF5		GCGCTAGCAGATCTCCATGAATCTACTCCTGATCC	NS*

*: nucleotide sequence

INDUSTRIAL UTILITY

According to the present invention, there are provided isolated human and mouse serine protease (hBSSP2 and mBSSP2) polynucleotides, their homologous forms, mature forms, precursors and polymorphic variants. Further, according to the present invention, there are provided hBSSP2 and mBSSP2 proteins as well as compositions containing hBSSP2 and mBssP2 polynucleotides and proteins, their production and use.

SEQUENCE LISTING FREE TEXT

SEQ ID NO: 11: Designed oligonucleotide to construct plasmid pSecTrypHis.

SEQ ID NO: 12: Designed oligonucleotide to construct plasmid pSecTrypHis.

SEQ ID NO: 13: Designed oligonucleotide primer to amplify neurosin-encoding sequence.

SEQ ID NO: 14: Designed oligonucleotide primer to amplify neurosin-encoding sequence.

SEQ ID NO: 15: Designed oligonucleotide primer to amplify a portion of plasmid pSecTrypHis/Neurosin.

SEQ ID NO: 16: Designed oligonucleotide primer to amplify a portion of plasmid pSecTrypHis/Neurosin.

SEQ ID NO: 17: Designed oligonucleotide primer to amplify a portion of plasmid pTrypHis.

SEQ ID NO: 18: Designed oligonucleotide primer to amplify a portion of plasmid pTrypSigTag.

SEQ ID NO: 19: Designed oligonucleotide primer to amplify a portion of plasmid pFBTrypSigTag.

SEQ ID NO: 20: Designed oligonucleotide primer to amplify conserved region of serine proteases-encoding sequence; n is a, c, g or t.

SEQ ID NO: 21: Designed oligonucleotide primer to amplify conserved region of serine proteases-encoding sequence; n is a, c, g or t.

SEQ ID NO: 22: Designed oligonucleotide primer designated as mBSSP2.0 for RACE for mBSSP2 (forward).

SEQ ID NO: 23: Designed oligonucleotide primer designated as mBSSP2.1 for RACE for mBSSP2 (forward).

SEQ ID NO: 24: Designed oligonucleotide primer designated as mBSSPF4 for RACE for mBSSP2 (forward).

SEQ ID NO: 25: Designed oligonucleotide primer designated as mBSSP2F5 to amplify mature mBSSP2-encoding region (forward).

SEQ ID NO: 26: Designed oligonucleotide primer designated as mBSSPF7 to amplify full-length mBSSP2-encoding mRNA (forward).

SEQ ID NO: 27: Designed oligonucleotide primer designated as mBSSP2.2 for RACE for mBSSP2 (reverse).

SEQ ID NO: 28: Designed oligonucleotide primer designated as mBSSP2R2 for RACE for mBSSP2 (reverse).

SEQ ID NO: 29: Designed oligonucleotide primer designated as mBSSP2R5/E to amplify full-length mBSSP2-encoding mRNA (reverse).

SEQ ID NO: 30: Designed oligonucleotide primer designated as BSSP2SPF to amplify a portion of hBSSP2 (forward).

SEQ ID NO: 31: Designed oligonucleotide primer designated as BSSP2SPR to amplify a portion of hBSSP2 (reverse).

SEQ ID NO: 32: Designed oligonucleotide primer designated as hBSSP2F11 for RACE for hBSSP2 (forward).

SEQ ID NO: 33: Designed oligonucleotide primer designated as hBSSP2R12 for RACE for hBSSP2 (reverse).

SEQ ID NO: 34: Designed oligonucleotide primer designated as hBSSP2R11 for RACE for hBSSP2 (reverse).

SEQ ID NO: 35: Designed oligonucleotide primer designated as hBSSP2F12 to amplify full length hBSSP2 (forward).

SEQ ID NO: 36: Designed oligonucleotide primer designated as hBSSP2R13/E to amplify full length hBSSP2 (reverse).

SEQ ID NO: 37: Designed oligonucleotide primer for RACE for hBSSP2.

SEQ ID NO: 38: Designed oligonucleotide primer designated as hBSSP2F13 to amplify a portion of hBSSP2 (forward).

SEQ ID NO: 39: Designed oligonucleotide primer designated as FBTrpsigtagF5 to detect hBSSP2.

SEQ ID NO: 40: Designed oligonucleotide to construct plasmid pTrypHis.

SEQ ID NO: 41: Designed oligonucleotide to construct plasmid pTrypHis.

What is claimed is:

1. A protein having the amino acid sequence of 238 amino acids represented by SEQ ID NO: 2; or a protein having an amino acid sequence derived from the amino acid sequence represented by SEQ ID NO: 2 by deletion, substitution or addition of one to several amino acids and having the same property as that of the protein having the amino acid sequence represented by SEQ ID NO: 2; or a modified derivative thereof.

2. A nucleotide sequence represented by the 1st to 714th bases of SEQ ID NO: 1; a nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 2; or a nucleotide sequence hybridizable with a nucleotide sequence which is complementary to the above nucleotide sequence under stringent conditions and encoding a protein having the same property as that of the protein having the amino acid sequence represented by SEQ ID NO: 2.

3. A protein having the amino acid sequence of 273 amino acids represented by SEQ ID NO: 4; or a protein having an amino acid sequence derived from the amino acid sequence represented by SEQ ID NO: 4 by deletion, substitution or addition of one to several amino acids and having the same property as that of the protein having the amino acid sequence represented by SEQ ID NO: 4; or a modified derivative thereof.

4. A nucleotide sequence represented by the 247th to 1065th bases of SEQ ID NO: 3; a nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 4; or a nucleotide sequence hybridizable with a nucleotide sequence which is complementary to the above nucleotide sequence under stringent conditions and encoding a protein having the same property as that of the protein having the amino acid sequence represented by SEQ ID NO: 4.

5. A protein having the amino acid sequence of 311 amino acids represented by SEQ ID NO: 6; or a protein having an amino acid sequence derived from the amino acid sequence represented by SEQ ID NO: 6 by deletion, substitution or addition of one to several amino acids and having the same property as that of the protein having the amino acid sequence represented by SEQ ID NO: 6; or a modified derivative thereof.

6. A nucleotide sequence represented by the 516th to 1448th bases of SEQ ID NO: 5; a nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 6; or a nucleotide sequence hybridizable with a nucleotide sequence which is complementary to the above nucleotide sequence under stringent conditions and encoding a protein having the same property as that of the protein having the amino acid sequence represented by SEQ ID NO: 6.

7. A protein having the amino acid sequence of

455 amino acids represented by SEQ ID NO: 8; or a protein having an amino acid sequence derived from the amino acid sequence represented by SEQ ID NO: 8 by deletion, substitution or addition of one to several amino acids and having the same property as that of the protein having the amino acid sequence represented by SEQ ID NO: 8; or a modified derivative thereof.

8. A nucleotide sequence represented by the 116th to 1450th bases of SEQ ID NO: 7; a nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 8; or a nucleotide sequence hybridizable with a nucleotide sequence which is complementary to the above nucleotide sequence under stringent conditions and encoding a protein having the same property as that of the protein having the amino acid sequence represented by SEQ ID NO: 8.

9. A protein having the amino acid sequence of 240 amino acids represented by the 1st to 240th amino acids of SEQ ID NO: 10; or a protein having an amino acid sequence derived from the amino acid sequence represented by the 1st to 240th amino acids of SEQ ID NO: 10 by deletion, substitution or addition of one to several amino acids and having the same property as that of the protein having the amino acid sequence represented by the 1st to 240th amino acids of SEQ ID NO: 10; or a modified derivative thereof.

10. A nucleotide sequence represented by the 807th to 1526th bases of SEQ ID NO: 9; a nucleotide sequence encoding the amino acid sequence represented by the 1st to 240th amino acids of SEQ ID NO: 10; or a nucleotide sequence hybridizable with a nucleotide sequence which is complementary to the above nucleotide sequence under stringent conditions and encoding a protein having the same property as that of the protein having the amino acid sequence represented by the 1st to 240th amino acids of SEQ ID NO: 10.

11. A protein having the amino acid sequence of 457 amino acids represented by the -217th to 240th amino acids of SEQ ID NO: 10; or a protein having an amino acid sequence derived from the amino acid sequence represented by the -217th to 240th amino acids of SEQ ID NO: 10 by deletion, substitution or addition of one to several amino acids and having the same property as that of the protein having the amino acid sequence represented by the -217th to 240th amino acids of SEQ ID NO: 10; or a modified derivative thereof.

12. A nucleotide sequence represented by the 156th to 1526th bases of SEQ ID NO: 9; a nucleotide sequence encoding the amino acid sequence represented by the -217th to 240th amino acids of SEQ ID NO: 10; or a nucleotide sequence hybridizable with a nucleotide sequence

which is complementary to the above nucleotide sequence under stringent conditions and encoding a protein having the same property as that of the protein having the amino acid sequence represented by the -217th to 240th amino acids of SEQ ID NO: 10.

13. A protein having the amino acid sequence of 217 amino acids represented by the -217th to -1st amino acids of SEQ ID NO: 10; or a protein having an amino acid sequence derived from the amino acid sequence represented by the -217th to -1st amino acids of SEQ ID NO: 10 by deletion, substitution or addition of one to several amino acids and having the same property as that of the protein having the amino acid sequence represented by the -217th to -1st amino acids of SEQ ID NO: 10; or a modified derivative thereof.

14. A nucleotide sequence represented by the 156th to 806th bases of SEQ ID NO: 9; a nucleotide sequence encoding the amino acid sequence represented by the -217th to -1st amino acids of SEQ ID NO: 10; or a nucleotide sequence hybridizable with a nucleotide sequence which is complementary to the above nucleotide sequence under stringent conditions and encoding a protein having the same property as that of the protein having the amino acid sequence represented by the -217th to -1st amino acids of SEQ ID NO: 10.

15. A nucleotide sequence represented by SEQ ID NO: 1; or a nucleotide sequence hybridizable with a nucleotide sequence which is complementary to the above nucleotide sequence under stringent conditions and encoding a protein having the same property as that of the protein encoded by the nucleotide sequence represented by SEQ ID NO: 1.

16. A nucleotide sequence represented by SEQ ID NO: 3; or a nucleotide sequence hybridizable with a nucleotide sequence which is complementary to the above nucleotide sequence under stringent conditions and encoding a protein having the same property as that of the protein encoded by the nucleotide sequence represented by SEQ ID NO: 3.

17. A nucleotide sequence represented by SEQ ID NO: 5; or a nucleotide sequence hybridizable with a nucleotide sequence which is complementary to the above nucleotide sequence under stringent conditions and encoding a protein having the same property as that of the protein encoded by the nucleotide sequence represented by SEQ ID NO: 5.

18. A nucleotide sequence represented by SEQ ID NO: 7; or a nucleotide sequence hybridizable with a nucleotide sequence which is complementary to the above nucleotide sequence under stringent conditions and encoding

a protein having the same property as that of the protein encoded by the nucleotide sequence represented by SEQ ID NO: 7.

19. A nucleotide sequence represented by SEQ ID NO: 9; or a nucleotide sequence hybridizable with a nucleotide sequence which is complementary to the above nucleotide sequence under stringent conditions and encoding a protein having the same property as that of the protein encoded by the nucleotide sequence represented by SEQ ID NO: 9.

20. A vector comprising the nucleotide sequence according to any one of claims 2, 4, 6, 8, 10, 12 and 14-19.

21. Transformed cells having the nucleotide sequence according to any one of claims 2, 4, 6, 8, 10, 12 and 14-19 in an expressible state.

22. A process for producing a protein which comprises culturing cells transformed with the nucleotide sequence according to any one of claims 2, 4, 6, 8, 15-18, and collecting mBSSP2 produced.

23. A process for producing a protein which comprises culturing cells transformed with the nucleotide sequence according to any one of claims 10, 12, 14 or 19, and collecting hBSSP2 produced.

24. The process according to claim 22 or 23, wherein the cells are *E. coli* cells, animal cells or insect

cells.

25. A non-human transgenic animal whose expression level of BSSP2 gene has been altered.

26. The non-human transgenic animal according to claim 25, wherein BSSP2 gene is cDNA, genomic DNA or synthetic DNA encoding BSSP2.

27. The non-human transgenic animal according to claim 25, wherein the expression level has been altered by mutating a gene expression regulatory site.

28. A knockout mouse whose BSSP2 gene function is deficient.

29. An antibody against the protein according to any one of claims 1, 3, 5, 7, 9, 11 and 13 or a fragment thereof.

30. The antibody according to claim 29 which is a polyclonal antibody, a monoclonal antibody or a peptide antibody.

31. A process for producing a monoclonal antibody against the protein according to any one of claims 1, 3, 5, 7, 9, 11 and 13 or a fragment thereof which comprises administering the protein according to any one of claims 1, 3, 5, 7, 9, 11 and 13 or a fragment thereof to a warm-blooded animal other than a human being, selecting the animal whose antibody titer is recognized, collecting its spleen or lymph node, fusing the antibody producing cells

contained therein with myeloma cells to prepare a monoclonal antibody producing hybridoma.

32. A method for determining the protein according to any one of claims 1, 3, 5, 7, 9, 11 and 13 or a fragment thereof in a specimen which is based on immunological binding of an antigen against the protein or a fragment thereof to the protein or a fragment thereof in the specimen.

33. A method for determining hBSSP2 or a fragment thereof in a specimen which comprises reacting a monoclonal antibody or a polyclonal antibody against the protein according to any one of claims 9, 11 and 13 or a fragment thereof and a labeled antibody with hBSSP2 or a fragment thereof in the specimen to detect a sandwich complex produced.

34. A method for determining hBSSP2 or a fragment thereof in a specimen which comprises reacting a monoclonal antibody or a polyclonal antibody against the protein according to any one of claims 9, 11 and 13 and a fragment thereof with labeled hBBSP2 and hBSSP2 or a fragment thereof in the specimen competitively to detect an amount of hBSSP2 or a fragment thereof in the specimen based on an amount of the labeled hBBSP2 reacted with the antibody.

35. The method according to any one of claims

32-34, wherein the specimen is a body fluid.

36. A diagnostic marker for diseases in tissues comprising the protein according to any one of claims 1, 3, 5, 7, 9, 11 and 13.

37. The marker according to claim 36 to be used for diagnosis of Alzheimer's disease or epilepsy in brain.

38. The marker according to claim 36 to be used for diagnosis of cancer or inflammation of brain, prostate or testicle.

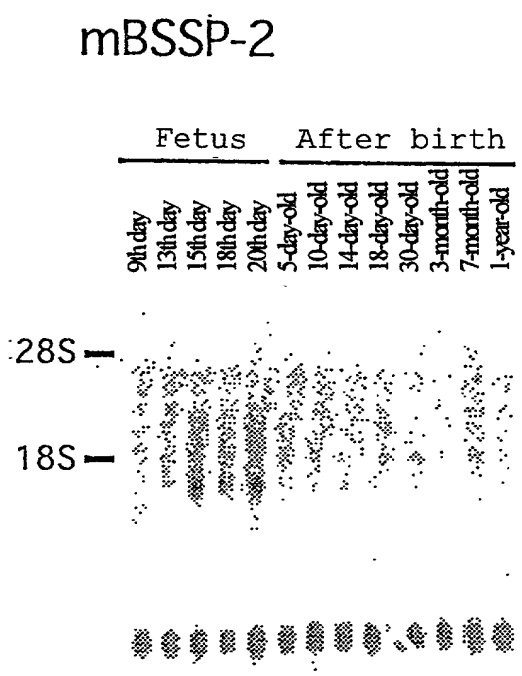
39. The marker according to claim 36 to be used for diagnosis of sterility in semen or sperms

40. The marker according to claim 36 to be used for diagnosis of prostatic hypertrophy in prostate.

Abstract of the disclosure:

There are provided proteins having the amino acid sequences represented by SEQ ID NOS: 2, 4, 6, 8 and 10; proteins having amino acid sequences derived from these amino acid sequences by deletion, substitution or addition of one to several amino acids; and nucleotide sequences encoding the same; transgenic non-human animals with altered expression level of a serine protease BSSP2; an antibody against BSSP2; and a method for detecting BSSP2 in a specimen by using the antibody.

Fig. 1



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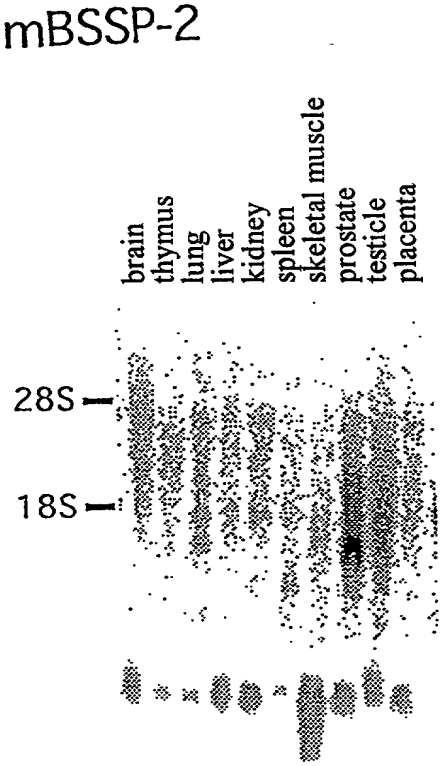
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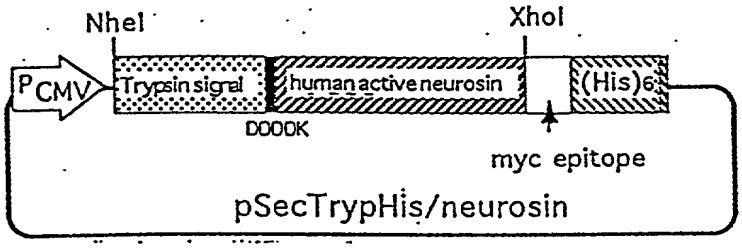
Fig. 3



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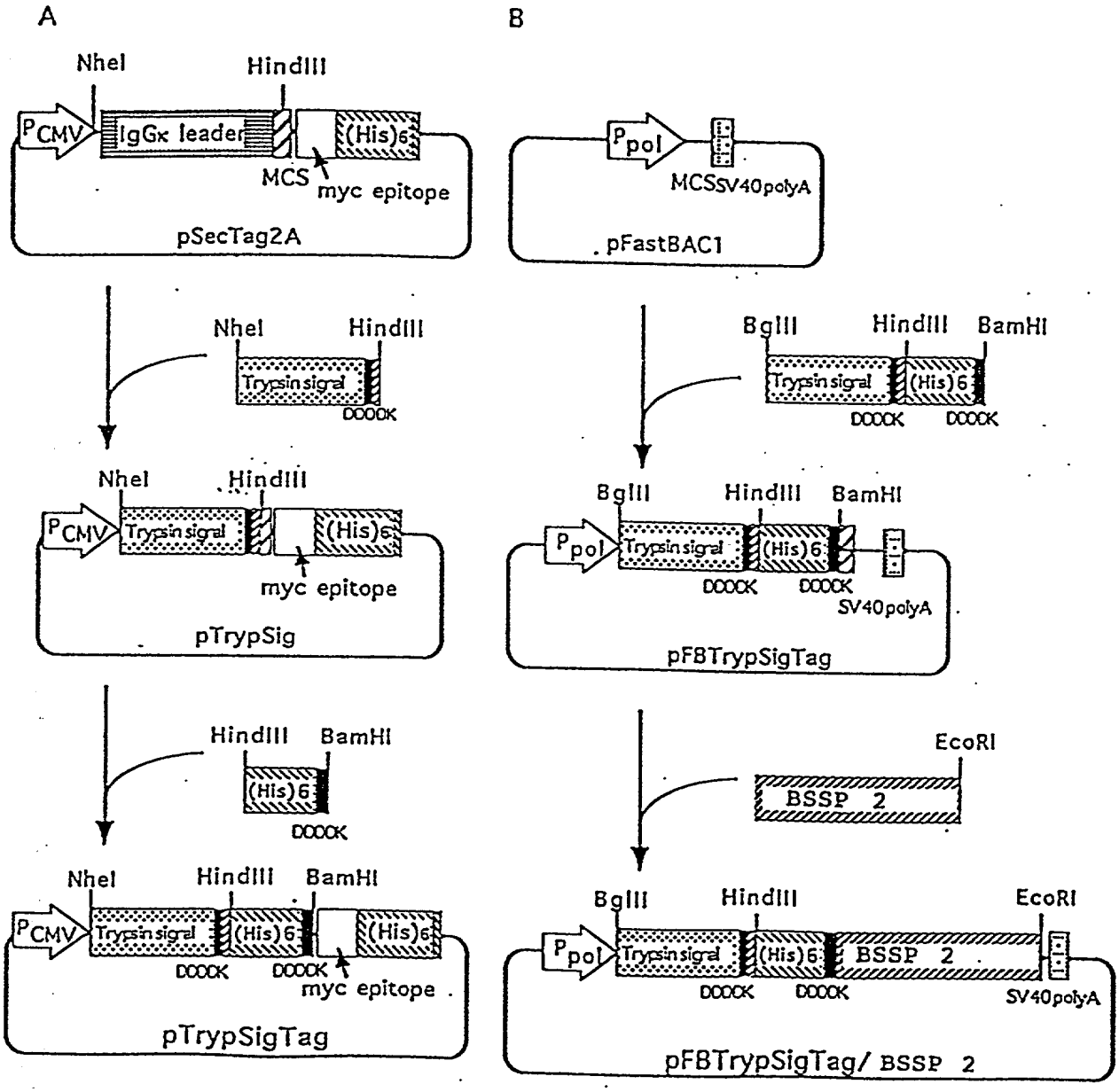
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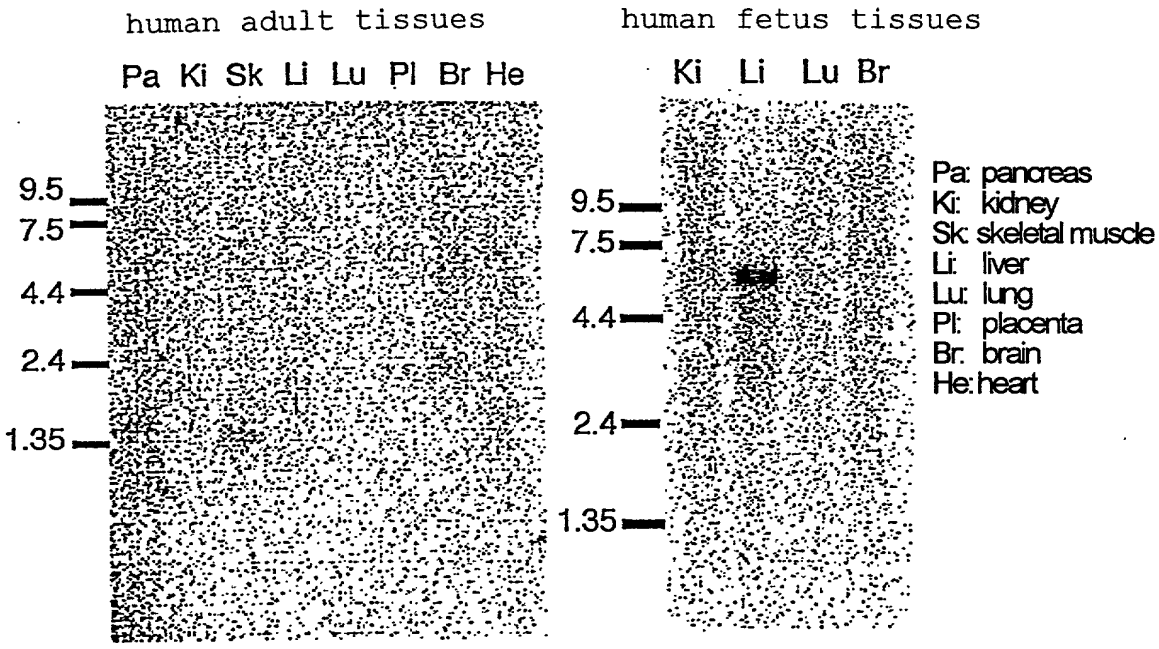

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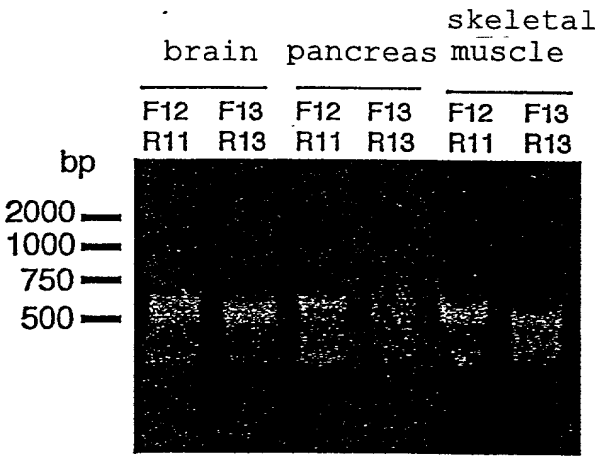

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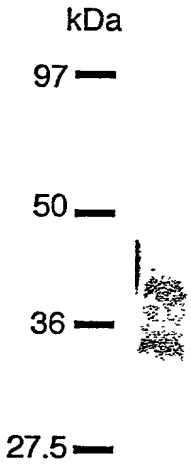

Page 1 of 2 Pages [x] Original [ ] Supplemental Atty. Docket:

Combined Declaration for Patent Application and Power of Attorney

As a below-named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name; and that I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

NOVEL SERINE PROTEASE BSSP2

the specification of which (check one)

[ ] is attached hereto;
[ ] was filed in the United States under 35 U.S.C. §111 on _____, as U.S. Appln. No. _____*; or
[X] was/will be filed in the U.S. under 35 U.S.C. §371 by entry into the U.S. national stage of an international (PCT) application, PCT/JP99/06475; filed 19/11/1999, entry requested on _____*; national stage application received U.S. Appln. No. _____*; §371/§102(e) date _____* (* if known)

and was amended on _____ (if applicable).
(include dates of amendments under PCT Art. 19 and 34 if PCT)

I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above; and I acknowledge the duty to disclose to the Patent and Trademark Office (PTO) all information known by me to be material to patentability as defined in 37 C.F.R. §1.56.

I hereby claim foreign priority benefits under 35 U.S.C. §§ 119 and 365 of any prior foreign application(s) for patent or inventor's certificate, or prior PCT application(s) designating a country other than the U.S., listed below with the "Yes" box checked and have also identified below any such application having a filing date before that of the application on which priority is claimed:

347785/1998 (Number)	Japan (Country)	20/11/1998 (Day Month Year Filed)	[X] YES	[ ] NO
_____ (Number)	_____ (Country)	_____ (Day Month Year Filed)	[ ] YES	[ ] NO

I hereby claim the benefit under 35 U.S.C. §120 of any prior U.S. non-provisional application(s) or prior PCT application(s) designating the U.S. listed below, or under §119(e) of any prior U.S. provisional applications listed below, and, insofar as the subject matter of each of the claims of this application is not disclosed in such U.S. or PCT application in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose to the PTO all information as defined in 37 C.F.R. §1.56(a) which occurred between the filing date of the prior application and the national filing date of this application:

_____ (Application No.)	_____ (Day Month Year Filed)	_____ (Status: patented, pending, abandoned)
_____ (Application No.)	_____ (Day Month Year Filed)	_____ (Status: patented, pending, abandoned)
_____ (Application No.)	_____ (Day Month Year Filed)	_____ (Status: patented, pending, abandoned)

As a named inventor, I hereby appoint the following registered practioners to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

All of the practioners associated with Customer Number 001444

Direct all correspondence to the address associated with **Customer Number 001444**; i.e.,

BROWDY AND NEIMARK, P.L.L.C.
624 Ninth Street, N.W.
Washington, D.C. 20001-5303
(202) 628-5197

The undersigned hereby authorizes the U.S. Attorneys or Agents appointed herein to accept and follow instructions from AOYAMA & PARTNERS as to any action to be taken in the U.S. Patent and Trademark Office regarding this application without direct communication between the U.S. Attorneys or Agents and the undersigned. In the event of a change of the persons from whom instructions may be taken, the U.S. Attorneys or Agents appointed herein will be so notified by the undersigned.

Title: NOVEL SERINE PROTEASE BSSP2

Atty. Docket:

U.S. Application filed _____, Serial No. _____

PCT Application filed Nov. 19, 1999, Serial No. PCT/JP99/06475

I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. §1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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FULL NAME OF SIXTH JOINT INVENTOR	INVENTOR'S SIGNATURE	DATE
RESIDENT		CITIZENSHIP
POST OFFICE ADDRESS		

FULL NAME OF SEVENTH JOINT INVENTOR	INVENTOR'S SIGNATURE	DATE
RESIDENT		CITIZENSHIP
POST OFFICE ADDRESS		

ALL INVENTORS MUST REVIEW APPLICATION AND DECLARATION BEFORE SIGNING. ALL ALTERATIONS MUST BE INITIALED AND DATED BY ALL INVENTORS PRIOR TO EXECUTION. NO ALTERATIONS CAN BE MADE AFTER THE DECLARATION IS SIGNED. ALL PAGES OF DECLARATION MUST BE SEEN BY ALL INVENTORS.

SEQUENCE LISTING

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Tyr Ser Ala Gln Asn His Asp Tyr Asp Val Ala Leu Leu Gln Leu Arg Thr
          85                  90                  95
cca atc aac ttc tca gac acc gtg gac gct gtg tgc ttg ccg gcc aag gag  699
Pro Ile Asn Phe Ser Asp Thr Val Asp Ala Val Cys Leu Pro Ala Lys Glu
100                 105                 110                 115
cag tac ttt cca tgg ggg tcg cag tgc tgg gtg tct ggc tgg ggc cac acc  750
Gln Tyr Phe Pro Trp Gly Ser Gln Cys Trp Val Ser Gly Trp Gly His Thr
               120                 125                 130
gac ccc agc cat act cat agc tca gat aca ctg cag gac aca atg gta ccc  801
Asp Pro Ser His Thr His Ser Ser Asp Thr Leu Gln Asp Thr Met Val Pro
    135                 140                 145                 150
ctg ctc agc acc cac ctc tgc aac agc tca tgc atg tac agt ggg gca ctt  852
Leu Leu Ser Thr His Leu Cys Asn Ser Ser Cys Met Tyr Ser Gly Ala Leu
                    155                 160                 165
aca cac cgc atg ttg tgt gct ggc tac ctg gat gga agg gca gac gca tgc  903
Thr His Arg Met Leu Cys Ala Gly Tyr Leu Asp Gly Arg Ala Asp Ala Cys
          170                 175                 180
cag gga gac agc ggg gga ccc ctg gta tgt ccc agt ggt gac acg tgg cac  954
Gln Gly Asp Ser Gly Gly Pro Leu Val Cys Pro Ser Gly Asp Thr Trp His
185                 190                 195                 200
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ctt gta ggg gtg gtc agc tgg ggt cgt ggc tgt gca gag ccc aat cgc cca 1005
Leu Val Gly Val Val Ser Trp Gly Arg Gly Cys Ala Glu Pro Asn Arg Pro
205 210 215

ggt gtc tat gcc aag gta gca gag ttc ctg gac tgg atc cat gac act gtg 1056
Gly Val Tyr Ala Lys Val Ala Glu Phe Leu Asp Trp Ile His Asp Thr Val
220 225 230 235

cag gtc cgc tagccgaaga agcagcagca gccacctgtg acgccgagct gtggatcgcc 1115
Gln Val Arg

catggatcac cccagtctgg gggccagcat ctgggtcact gggcctctcc ccaaaggctc 1175

tgacttcgag ttcatctttc tcatctgaga acctccacaa caggaaaagg agtctgcggc 1235

tagattggga atgatggtga gaggaaggga taggaggaca gaagagacag cagaggcttc 1295

tggaagcatc tgggagactg ctcctctgct ccccccacac cccacgtgca tccactgggg 1355

gatgctggag atgcccaatc cttgtttctt gtggggccac tggaaggcta agtccaactt 1415

tagaggatgc cctgtctcga gagttactag gcagataagg ttaaggttgg acaagctcag 1475

gtaaaggcac ggaagtcaag atcccctctc ccccgtgcgg tcctgttctg aggtaagcta 1535

atagccccgc accaggcaga ggtctacagg gtaagaagga tgcagttggg ctacacgacg 1595

ctatttttca aatgatgttt ctgtaaattg gttgagagag ttttgttatt aaacagaaat 1655

tatgtataaa aaaaaaaaaa aaaaaaaaaa 1685

<210> 4

<211> 273

<212> PRT

<213> mouse

<400> 4

Met Glu Ala Gln Val Gly Leu Leu Trp Val Ser Ala Asn Cys Pro
-35 -30 -25

Ser Gly Arg Ile Val Ser Leu Lys Cys Ser Glu Cys Gly Ala Arg Pro Leu
-20 -15 -10 -5

Ala Ser Arg Ile Val Gly Gly Gln Ala Val Ala Ser Gly Arg Trp Pro Trp
-1 1 5 10

Gln Ala Ser Val Met Leu Gly Ser Arg His Thr Cys Gly Ala Ser Val Leu
15 20 25 30

Ala Pro His Trp Val Val Thr Ala Ala His Cys Met Tyr Ser Phe Arg Leu
35 40 45

Ser Arg Leu Ser Ser Trp Arg Val His Ala Gly Leu Val Ser His Gly Ala
50 55 60 65

Val Arg Gln His Gln Gly Thr Met Val Glu Lys Ile Ile Pro His Pro Leu
70 75 80

Tyr Ser Ala Gln Asn His Asp Tyr Asp Val Ala Leu Leu Gln Leu Arg Thr
85 90 95

Pro Ile Asn Phe Ser Asp Thr Val Asp Ala Val Cys Leu Pro Ala Lys Glu
100 105 110 115

Gln Tyr Phe Pro Trp Gly Ser Gln Cys Trp Val Ser Gly Trp Gly His Thr
120 125 130

Asp Pro Ser His Thr His Ser Ser Asp Thr Leu Gln Asp Thr Met Val Pro
135 140 145 150

Leu Leu Ser Thr His Leu Cys Asn Ser Ser Cys Met Tyr Ser Gly Ala Leu
155 160 165

Thr His Arg Met Leu Cys Ala Gly Tyr Leu Asp Gly Arg Ala Asp Ala Cys
170 175 180
Gln Gly Asp Ser Gly Gly Pro Leu Val Cys Pro Ser Gly Asp Thr Trp His
185 190 195 200
Leu Val Gly Val Val Ser Trp Gly Arg Gly Cys Ala Glu Pro Asn Arg Pro
205 210 215
Gly Val Tyr Ala Lys Val Ala Glu Phe Leu Asp Trp Ile His Asp Thr Val
220 225 230 235
Gln Val Arg

<210> 5

<211> 2068

<212> DNA

<213> mouse

<400> 5

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acttactgcc ttatatcagt gcagctgact catatgagtc caacactgga tgaccaaagc 120

ccaatggaga ttcggtgcac ggaagagggt gctgggcctg ggatcttcag aatggagttg 180

ggagaccaga ggcaatccat ttctcagtcc caacgctggt gctgcctgca acgtggctgt 240

gtaatactgg gcgtcctggg gctgctggct ggagcaggca ttgcttcatg gctcttagtg 300

ttgtatctat ggccggctgc ctctccatcc atctctggga cgttgcagga ggaggagatg 360

actttgaact gtccaggagt gagctgtgag gaagagctcc ttccatctct tcccaaaaca 420

gaataaatgg aggggatctt ctgcttcaag tacaagtaag agctcggcca gactggctcc 480

tggtctgcca tgagggctgg agccccgccc tgggc atg cac atc tgc aag agt ctt 536

Met His Ile Cys Lys Ser Leu

-70

ggg cat atc agg ctt act caa cac aag gcc gtg aat ctg tct gac atc aag 587

Gly His Ile Arg Leu Thr Gln His Lys Ala Val Asn Leu Ser Asp Ile Lys

-65 -60 -55 -50

ctc aac aga tcc cag gag ttt gct caa ctc tct gct aga ccg gga ggc ctt 638

Leu Asn Arg Ser Gln Glu Phe Ala Gln Leu Ser Ala Arg Pro Gly Gly Leu

-45 -40 -35

gta gag gag gca tgg aag ccc agc gct aac tgt cct tct ggc cga att gtt 689

Val Glu Glu Ala Trp Lys Pro Ser Ala Asn Cys Pro Ser Gly Arg Ile Val

-30 -25 -20

tct ctc aaa tgt tct gag tgt ggg gca agg cct ctg gct tct cga ata gtt 740

Ser Leu Lys Cys Ser Glu Cys Gly Ala Arg Pro Leu Ala Ser Arg Ile Val

-15 -10 -5 -1 1

ggc ggc caa gct gtg gct tct ggg cgc tgg cca tgg caa gct agc gtg atg 791

Gly Gly Gln Ala Val Ala Ser Gly Arg Trp Pro Trp Gln Ala Ser Val Met

5 10 15

ctt ggc tcc cgg cac acg tgt ggg gcc tct gtg ttg gca cca cac tgg gta 842

Leu Gly Ser Arg His Thr Cys Gly Ala Ser Val Leu Ala Pro His Trp Val

20 25 30 35

gtg act gct gcc cac tgc atg tac agt ttc agg ctg tcc cgc cta tcc agc 893

Val Thr Ala Ala His Cys Met Tyr Ser Phe Arg Leu Ser Arg Leu Ser Ser

40 45 50

tgg cgg gtt cat gca ggg ctg gtc agc cat ggt gct gtc cga caa cac cag 944

Trp Arg Val His Ala Gly Leu Val Ser His Gly Ala Val Arg Gln His Gln

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     55                  60                  65                  70
gga act atg gtg gag aag atc att cct cat cct ttg tac agt gcc cag aac  995
Gly Thr Met Val Glu Lys Ile Ile Pro His Pro Leu Tyr Ser Ala Gln Asn
                 75                  80                  85
cat gac tat gat gtg gct ctg ctg cag ctc cgg aca cca atc aac ttc tca 1046
His Asp Tyr Asp Val Ala Leu Leu Gln Leu Arg Thr Pro Ile Asn Phe Ser
          90                  95                 100
gac acc gtg gac gct gtg tgc ttg ccg gcc aag gag cag tac ttt cca tgg 1097
Asp Thr Val Asp Ala Val Cys Leu Pro Ala Lys Glu Gln Tyr Phe Pro Trp
105                 110                 115                 120
ggg tcg cag tgc tgg gtg tct ggc tgg ggc cac acc gac ccc agc cat act 1148
Gly Ser Gln Cys Trp Val Ser Gly Trp Gly His Thr Asp Pro Ser His Thr
            125                 130                 135
cat agc tca gat aca ctg cag gac aca atg gta ccc ctg ctc agc acc cac 1199
His Ser Ser Asp Thr Leu Gln Asp Thr Met Val Pro Leu Leu Ser Thr His
    140                 145                 150                 155
ctc tgc aac agc tca tgc atg tac agt ggg gca ctt aca cac cgc atg ttg 1250
Leu Cys Asn Ser Ser Cys Met Tyr Ser Gly Ala Leu Thr His Arg Met Leu
                160                 165                 170
tgt gct ggc tac ctg gat gga agg gca gac gca tgc cag gga gac agc ggg 1301
Cys Ala Gly Tyr Leu Asp Gly Arg Ala Asp Ala Cys Gln Gly Asp Ser Gly
        175                 180                 185
gga ccc ctg gta tgt ccc agt ggt gac acg tgg cac ctt gta ggg gtg gtc 1352
Gly Pro Leu Val Cys Pro Ser Gly Asp Thr Trp His Leu Val Gly Val Val
190                 195                 200                 205
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agc tgg ggt cgt ggc tgt gca gag ccc aat cgc cca ggt gtc tat gcc aag 1403
Ser Trp Gly Arg Gly Cys Ala Glu Pro Asn Arg Pro Gly Val Tyr Ala Lys
210 215 220

gta gca gag ttc ctg gac tgg atc cat gac act gtg cag gtc cgc tagccga 1455
Val Ala Glu Phe Leu Asp Trp Ile His Asp Thr Val Gln Val Arg
225 230 235

agaagcagca gcagccacct gtgacgccga gctgtggatc gcccatggat caccccagtc 1515

tgggggccag catctgggtc actgggcctc tccccaaagg ctctgacttc gagttcatct 1575

ttctcatctg agaacctcca caacaggaaa aggagtctgc ggctagattg ggaatgatgg 1635

tgagaggaag ggataggagg acagaagaga cagcagaggc ttctggaagc atctgggaga 1695

ctgctcctct gctcccccca caccccacgt gcatccactg ggggatgctg gagatgccca 1755

atccttgttt cttgtggggc cactggaagg ctaagtccaa ctttagagga tgccctgtct 1815

cgagagttac taggcagata aggttaaggt tggacaagct caggtaaagg cacggaagtc 1875

aagatcccct ctcccccgtg cggtcctgtt ctgaggtaag ctaatagccc cgcaccaggc 1935

agaggtctac agggtaagaa ggatgcagtt gggctacacg acgctatttt tcaaatgatg 1995

tttctgtaaa ttggttgaga gagttttgtt attaaacaga aattatgtat aaaaaaaaaa 2055

aaaaaaaaaa aaa 2068

<210> 6

<211> 311

<212> PRT

<213> mouse

<400> 6

Met His Ile Cys Lys Ser Leu

-70

Gly His Ile Arg Leu Thr Gln His Lys Ala Val Asn Leu Ser Asp Ile Lys

-65 -60 -55 -50

Leu Asn Arg Ser Gln Glu Phe Ala Gln Leu Ser Ala Arg Pro Gly Gly Leu

-45 -40 -35

Val Glu Glu Ala Trp Lys Pro Ser Ala Asn Cys Pro Ser Gly Arg Ile Val

-30 -25 -20

Ser Leu Lys Cys Ser Glu Cys Gly Ala Arg Pro Leu Ala Ser Arg Ile Val

-15 -10 -5 -1 1

Gly Gly Gln Ala Val Ala Ser Gly Arg Trp Pro Trp Gln Ala Ser Val Met

5 10 15

Leu Gly Ser Arg His Thr Cys Gly Ala Ser Val Leu Ala Pro His Trp Val

20 25 30 35

Val Thr Ala Ala His Cys Met Tyr Ser Phe Arg Leu Ser Arg Leu Ser Ser

40 45 50

Trp Arg Val His Ala Gly Leu Val Ser His Gly Ala Val Arg Gln His Gln

55 60 65 70

Gly Thr Met Val Glu Lys Ile Ile Pro His Pro Leu Tyr Ser Ala Gln Asn

75 80 85

His Asp Tyr Asp Val Ala Leu Leu Gln Leu Arg Thr Pro Ile Asn Phe Ser

90 95 100

Asp Thr Val Asp Ala Val Cys Leu Pro Ala Lys Glu Gln Tyr Phe Pro Trp

105 110 115 120

Gly Ser Gln Cys Trp Val Ser Gly Trp Gly His Thr Asp Pro Ser His Thr

125 130 135

His Ser Ser Asp Thr Leu Gln Asp Thr Met Val Pro Leu Leu Ser Thr His
140 145 150 155

Leu Cys Asn Ser Ser Cys Met Tyr Ser Gly Ala Leu Thr His Arg Met Leu
160 165 170

Cys Ala Gly Tyr Leu Asp Gly Arg Ala Asp Ala Cys Gln Gly Asp Ser Gly
175 180 185

Gly Pro Leu Val Cys Pro Ser Gly Asp Thr Trp His Leu Val Gly Val Val
190 195 200 205

Ser Trp Gly Arg Gly Cys Ala Glu Pro Asn Arg Pro Gly Val Tyr Ala Lys
210 215 220

Val Ala Glu Phe Leu Asp Trp Ile His Asp Thr Val Gln Val Arg
225 230 235

<210> 7

<211> 2070

<212> DNA

<213> mouse

<400> 7

cccagcagaa cttactgcct tatatcagtg cagctgactc atatgccctg gtgtggggct 60

gctggatctt caaccactat ttctccagag tccaacactg gatgaccaaa gccca atg 118
Met

gag att cgg tgc acg gaa gag ggt gct ggg cct ggg atc ttc aga atg gag 169
Glu Ile Arg Cys Thr Glu Glu Gly Ala Gly Pro Gly Ile Phe Arg Met Glu

-205 -200 -195 -190

ttg gga gac cag agg caa tcc att tct cag tcc caa cgc tgg tgc tgc ctg 220

Leu Gly Asp Gln Arg Gln Ser Ile Ser Gln Ser Gln Arg Trp Cys Cys Leu

-185 -180 -175

caa cgt ggc tgt gta ata ctg ggc gtc ctg ggg ctg ctg gct gga gca ggc 271

Gln Arg Gly Cys Val Ile Leu Gly Val Leu Gly Leu Leu Ala Gly Ala Gly

-170 -165 -160

att gct tca tgg ctc tta gtg ttg tat cta tgg cca gct gcc tct cca tcc 322

Ile Ala Ser Trp Leu Leu Val Leu Tyr Leu Trp Pro Ala Ala Ser Pro Ser

-155 -150 -145 -140

atc tct ggg acg ttg cag gag gag gag atg act ttg aac tgt cca gga gtg 373

Ile Ser Gly Thr Leu Gln Glu Glu Glu Met Thr Leu Asn Cys Pro Gly Val

-135 -130 -125

agc tgt gag gaa gag ctc ctt cca tct ctt ccc aaa aca gta tct ttc aga 424

Ser Cys Glu Glu Glu Leu Leu Pro Ser Leu Pro Lys Thr Val Ser Phe Arg

-120 -115 -110 -105

ata aat gga gag gat ctt ctg ctt caa gta caa gta aga gct cgg cca gac 475

Ile Asn Gly Glu Asp Leu Leu Leu Gln Val Gln Val Arg Ala Arg Pro Asp

-100 -95 -90

tgg ctc ctg gtc tgc cat gag ggc tgg agc ccc gcc ctg ggc atg cac atc 526

Trp Leu Leu Val Cys His Glu Gly Trp Ser Pro Ala Leu Gly Met His Ile

-85 -80 -75

tgc aag agt ctt ggg cat atc agg ctt act caa cac aag gcc gtg aat ctg 577

Cys Lys Ser Leu Gly His Ile Arg Leu Thr Gln His Lys Ala Val Asn Leu

-70 -65 -60 -55

tct gac atc aag ctc aac aga tcc cag gag ttt gct caa ctc tct gct aga 628
Ser Asp Ile Lys Leu Asn Arg Ser Gln Glu Phe Ala Gln Leu Ser Ala Arg
-50 -45 -40

ccg gga ggc ctt gta gag gag gca tgg aag ccc agc gct aac tgt cct tct 679
Pro Gly Gly Leu Val Glu Glu Ala Trp Lys Pro Ser Ala Asn Cys Pro Ser
-35 -30 -25 -20

ggc cga att gtt tct ctc aaa tgt tct gag tgt ggg gca agg cct ctg gct 730
Gly Arg Ile Val Ser Leu Lys Cys Ser Glu Cys Gly Ala Arg Pro Leu Ala
-15 -10 -5

tct cga ata gtt ggc ggc caa gct gtg gct tct ggg cgc tgg cca tgg caa 781
Ser Arg Ile Val Gly Gly Gln Ala Val Ala Ser Gly Arg Trp Pro Trp Gln
-1 1 5 10 15

gct agc gtg atg ctt ggc tcc cgg cac acg tgt ggg gcc tct gtg ttg gca 832
Ala Ser Val Met Leu Gly Ser Arg His Thr Cys Gly Ala Ser Val Leu Ala
20 25 30

cca cac tgg gta gtg act gct gcc cac tgc atg tac agt ttc agg ctg tcc 883
Pro His Trp Val Val Thr Ala Ala His Cys Met Tyr Ser Phe Arg Leu Ser
35 40 45

cgc cta tcc agc tgg cgg gtt cat gca ggg ctg gtc agc cat ggt gct gtc 934
Arg Leu Ser Ser Trp Arg Val His Ala Gly Leu Val Ser His Gly Ala Val
50 55 60 65

cga caa cac cag gga act atg gtg gag aag atc att cct cat cct ttg tac 985
Arg Gln His Gln Gly Thr Met Val Glu Lys Ile Ile Pro His Pro Leu Tyr
70 75 80

agt gcc cag aac cat gac tat gat gtg gct ctg ctg cag ctc cgg aca cca 1036

Ser Ala Gln Asn His Asp Tyr Asp Val Ala Leu Leu Gln Leu Arg Thr Pro

85 90 95 100

atc aac ttc tca gac acc gtg gac gct gtg tgc ttg ccg gcc aag gag cag 1087

Ile Asn Phe Ser Asp Thr Val Asp Ala Val Cys Leu Pro Ala Lys Glu Gln

105 110 115

tac ttt cca tgg ggg tcg cag tgc tgg gtg tct ggc tgg ggc cac acc gac 1138

Tyr Phe Pro Trp Gly Ser Gln Cys Trp Val Ser Gly Trp Gly His Thr Asp

120 125 130

ccc agc cat act cat agc tca gat aca ctg cag gac aca atg gta ccc ctg 1189

Pro Ser His Thr His Ser Ser Asp Thr Leu Gln Asp Thr Met Val Pro Leu

135 140 145 150

ctc agc acc cac ctc tgc aac agc tca tgc atg tac agt ggg gca ctt aca 1240

Leu Ser Thr His Leu Cys Asn Ser Ser Cys Met Tyr Ser Gly Ala Leu Thr

155 160 165

cac cgc atg ttg tgt gct ggc tac ctg gat gga agg gca gac gca tgc cag 1291

His Arg Met Leu Cys Ala Gly Tyr Leu Asp Gly Arg Ala Asp Ala Cys Gln

170 175 180 185

gga gac agc ggg gga ccc ctg gta tgt ccc agt ggt gac acg tgg cac ctt 1342

Gly Asp Ser Gly Gly Pro Leu Val Cys Pro Ser Gly Asp Thr Trp His Leu

190 195 200

gta ggg gtg gtc agc tgg ggt cgt ggc tgt gca gag ccc aat cgc cca ggt 1393

Val Gly Val Val Ser Trp Gly Arg Gly Cys Ala Glu Pro Asn Arg Pro Gly

205 210 215

gtc tat gcc aag gta gca gag ttc ctg gac tgg atc cat gac act gtg cag 1444

Val Tyr Ala Lys Val Ala Glu Phe Leu Asp Trp Ile His Asp Thr Val Gln

220 225 230 235

gtc cgc tagccgaaga agcagcagca gccacctgtg acgccgagct gtggatcgcc 1500

Val Arg

catggatcac cccagtctgg gggccagcat ctgggtcact gggcctctcc ccaaaggctc 1560

tgacttcgag ttcatctttc tcatctgaga acctccacaa caggaaaagg agtctgcggc 1620

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gtaaaggcac ggaagtcaag atcccctctc ccccgtgcgg tcctgttctg aggtaagcta 1920

atagccccgc accaggcaga ggtctacagg gtaagaagga tgcagttggg ctacacgacg 1980

ctatttttca aatgatgttt ctgtaaattg gttgagagag ttttgttatt aaacagaaat 2040

tatgtataaa aaaaaaaaaa aaaaaaaaaa 2070

<210> 8

<211> 445

<212> PRT

<213> mouse

<400> 8

Met

Glu Ile Arg Cys Thr Glu Glu Gly Ala Gly Pro Gly Ile Phe Arg Met Glu

-205 -200 -195 -190

Leu Gly Asp Gln Arg Gln Ser Ile Ser Gln Ser Gln Arg Trp Cys Cys Leu
-185 -180 -175

Gln Arg Gly Cys Val Ile Leu Gly Val Leu Gly Leu Leu Ala Gly Ala Gly
-170 -165 -160

Ile Ala Ser Trp Leu Leu Val Leu Tyr Leu Trp Pro Ala Ala Ser Pro Ser
-155 -150 -145 -140

Ile Ser Gly Thr Leu Gln Glu Glu Glu Met Thr Leu Asn Cys Pro Gly Val
-135 -130 -125

Ser Cys Glu Glu Glu Leu Leu Pro Ser Leu Pro Lys Thr Val Ser Phe Arg
-120 -115 -110 -105

Ile Asn Gly Glu Asp Leu Leu Leu Gln Val Gln Val Arg Ala Arg Pro Asp
-100 -95 -90

Trp Leu Leu Val Cys His Glu Gly Trp Ser Pro Ala Leu Gly Met His Ile
-85 -80 -75

Cys Lys Ser Leu Gly His Ile Arg Leu Thr Gln His Lys Ala Val Asn Leu
-70 -65 -60 -55

Ser Asp Ile Lys Leu Asn Arg Ser Gln Glu Phe Ala Gln Leu Ser Ala Arg
-50 -45 -40

Pro Gly Gly Leu Val Glu Glu Ala Trp Lys Pro Ser Ala Asn Cys Pro Ser
-35 -30 -25 -20

Gly Arg Ile Val Ser Leu Lys Cys Ser Glu Cys Gly Ala Arg Pro Leu Ala
-15 -10 -5

Ser Arg Ile Val Gly Gly Gln Ala Val Ala Ser Gly Arg Trp Pro Trp Gln
-1 1 5 10 15

Ala Ser Val Met Leu Gly Ser Arg His Thr Cys Gly Ala Ser Val Leu Ala

20 25 30

Pro His Trp Val Val Thr Ala Ala His Cys Met Tyr Ser Phe Arg Leu Ser

35 40 45

Arg Leu Ser Ser Trp Arg Val His Ala Gly Leu Val Ser His Gly Ala Val

50 55 60 65

Arg Gln His Gln Gly Thr Met Val Glu Lys Ile Ile Pro His Pro Leu Tyr

70 75 80

Ser Ala Gln Asn His Asp Tyr Asp Val Ala Leu Leu Gln Leu Arg Thr Pro

85 90 95 100

Ile Asn Phe Ser Asp Thr Val Asp Ala Val Cys Leu Pro Ala Lys Glu Gln

105 110 115

Tyr Phe Pro Trp Gly Ser Gln Cys Trp Val Ser Gly Trp Gly His Thr Asp

120 125 130

Pro Ser His Thr His Ser Ser Asp Thr Leu Gln Asp Thr Met Val Pro Leu

135 140 145 150

Leu Ser Thr His Leu Cys Asn Ser Ser Cys Met Tyr Ser Gly Ala Leu Thr

155 160 165

His Arg Met Leu Cys Ala Gly Tyr Leu Asp Gly Arg Ala Asp Ala Cys Gln

170 175 180 185

Gly Asp Ser Gly Gly Pro Leu Val Cys Pro Ser Gly Asp Thr Trp His Leu

190 195 200

Val Gly Val Val Ser Trp Gly Arg Gly Cys Ala Glu Pro Asn Arg Pro Gly

205 210 215

Val Tyr Ala Lys Val Ala Glu Phe Leu Asp Trp Ile His Asp Thr Val Gln

220 225 230 235

Val Arg

<210> 9

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<212> DNA

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<400> 9

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tgctgggctt gcatttaatc aatgcatggc cagagaacag gagcggaaca ttgcctagta 120

gaccctgagg ctttacaaca gtgctactga cccct 155

atg agc ctg atg ctg gat gac caa ccc cct atg gag gcc cag tat gca gag 206
Met Ser Leu Met Leu Asp Asp Gln Pro Pro Met Glu Ala Gln Tyr Ala Glu
-215 -210 -205

gag ggc cca gga cct ggg atc ttc aga gca gag cct gga gac cag cag cat 257
Glu Gly Pro Gly Pro Gly Ile Phe Arg Ala Glu Pro Gly Asp Gln Gln His
-200 -195 -190 -185

ccc att tct cag gcg gtg tgc tgg cgt tcc atg cga cgt ggc tgt gca gtg 308
Pro Ile Ser Gln Ala Val Cys Trp Arg Ser Met Arg Arg Gly Cys Ala Val
-180 -175 -170

ctg gga gcc ctg ggg ctg ctg gcc ggt gca ggt gtt ggc tca tgg ctc cta 359
Leu Gly Ala Leu Gly Leu Leu Ala Gly Ala Gly Val Gly Ser Trp Leu Leu
-165 -160 -155 -150

gtg ctg tat ctg tgt cct gct gcc tct cag ccc att tcc ggg acc ttg cag 410
Val Leu Tyr Leu Cys Pro Ala Ala Ser Gln Pro Ile Ser Gly Thr Leu Gln

-145 -140 -135

gat gag gag ata act ttg agc tgc tca gag gcc agc gct gag gaa gct ctg 461
Asp Glu Glu Ile Thr Leu Ser Cys Ser Glu Ala Ser Ala Glu Glu Ala Leu

-130 -125 -120

ctc cct gca ctc ccc aaa aca gta tct ttc aga ata aac agc gaa gac ttc 512
Leu Pro Ala Leu Pro Lys Thr Val Ser Phe Arg Ile Asn Ser Glu Asp Phe

-115 -110 -105 -100

ttg ctg gaa gcg caa gtg agg gat cag cca cgc tgg ctc ctg gtc tgc cat 563
Leu Leu Glu Ala Gln Val Arg Asp Gln Pro Arg Trp Leu Leu Val Cys His

-95 -90 -85

gag ggc tgg agc ccc gcc ctg ggg ctg cag atc tgc tgg agc ctt ggg cat 614
Glu Gly Trp Ser Pro Ala Leu Gly Leu Gln Ile Cys Trp Ser Leu Gly His

-80 -75 -70 -65

ctc aga ctc act cac cac aag gga gta aac ctc act gac atc aaa ctc aac 665
Leu Arg Leu Thr His His Lys Gly Val Asn Leu Thr Asp Ile Lys Leu Asn

-60 -55 -50

agt tcc cag gag ttt gct cag ctc tct cct aga ctg gga ggc ttc ctg gag 716
Ser Ser Gln Glu Phe Ala Gln Leu Ser Pro Arg Leu Gly Gly Phe Leu Glu

-45 -40 -35

gag gcg tgg cag ccc agg aac aac tgc act tct ggt caa gtt gtt tcc ctc 767
Glu Ala Trp Gln Pro Arg Asn Asn Cys Thr Ser Gly Gln Val Val Ser Leu

-30 -25 -20 -15

aga tgc tct gag tgt gga gcg agg ccc ctg gct tcc cgg ata gtt ggt ggg 818
Arg Cys Ser Glu Cys Gly Ala Arg Pro Leu Ala Ser Arg Ile Val Gly Gly

-10 -5 -1 1

cag tct gtg gct cct ggg cgc tgg ccg tgg cag gcc agc gtg gcc ctg ggc 869
Gln Ser Val Ala Pro Gly Arg Trp Pro Trp Gln Ala Ser Val Ala Leu Gly
5 10 15 20

ttc cgg cac acg tgt ggg ggc tct gtg cta gcg cca cgc tgg gtg gtg act 920
Phe Arg His Thr Cys Gly Gly Ser Val Leu Ala Pro Arg Trp Val Val Thr
25 30 35

gct gca cat tgt atg cac agt ttc agg ctg gcc cgc ctg tcc agc tgg cgg 971
Ala Ala His Cys Met His Ser Phe Arg Leu Ala Arg Leu Ser Ser Trp Arg
40 45 50 55

gtt cat gcg ggg ctg gtc agc cac agt gcc gtc agg ccc cac caa ggg gct 1022
Val His Ala Gly Leu Val Ser His Ser Ala Val Arg Pro His Gln Gly Ala
60 65 70

ctg gtg gag agg att atc cca cac ccc ctc tac agt gcc cag aat cat gac 1073
Leu Val Glu Arg Ile Ile Pro His Pro Leu Tyr Ser Ala Gln Asn His Asp
75 80 85

tac gac gtc gcc ctc ctg agg ctc cag acc gct ctc aac ttc tca gac act 1124
Tyr Asp Val Ala Leu Leu Arg Leu Gln Thr Ala Leu Asn Phe Ser Asp Thr
90 95 100 105

gtg ggc gct gtg tgc ctg ccg gcc aag gaa cag cat ttt ccg aag ggc tcg 1175
Val Gly Ala Val Cys Leu Pro Ala Lys Glu Gln His Phe Pro Lys Gly Ser
110 115 120

cgg tgc tgg gtg tct ggc tgg ggc cac acc cac cct agc cat act tac agc 1226
Arg Cys Trp Val Ser Gly Trp Gly His Thr His Pro Ser His Thr Tyr Ser
125 130 135 140

tcg gat atg ctc cag gac acg gtg gtg ccc ttg ttc agc act cag ctc tgc 1277

Ser Asp Met Leu Gln Asp Thr Val Val Pro Leu Phe Ser Thr Gln Leu Cys

145 150 155

aac agc tct tgc gtg tac agc gga gcc ctc acc ccc cgc atg ctt tgc gct 1328
Asn Ser Ser Cys Val Tyr Ser Gly Ala Leu Thr Pro Arg Met Leu Cys Ala

160 165 170

ggc tac ctg gac gga agg gct gat gca tgc cag gga gat agc ggg ggc ccc 1379
Gly Tyr Leu Asp Gly Arg Ala Asp Ala Cys Gln Gly Asp Ser Gly Gly Pro

175 180 185 190

cta gtg tgc cca gat ggg gac aca tgg cgc cta gtg ggg gtg gtc agc tgg 1430
Leu Val Cys Pro Asp Gly Asp Thr Trp Arg Leu Val Gly Val Val Ser Trp

195 200 205

ggg cgt gcg tgc gca gag ccc aat cac cca ggt gtc tac gcc aag gta gct 1481
Gly Arg Ala Cys Ala Glu Pro Asn His Pro Gly Val Tyr Ala Lys Val Ala

210 215 220 225

gag ttt ctg gac tgg atc cat gac act gct cag gac tcc ctc ctc 1526
Glu Phe Leu Asp Trp Ile His Asp Thr Ala Gln Asp Ser Leu Leu

230 235 240

tgagtcctgc tgtttcctcc agtctcactg cacaccactg cctcatgctt cctggggcct 1586

ccagcagctc cactaatgga ggagaggcag tagcctccga cacagaacgc atggacctcc 1646

tactactgtg tgtgaggaac agtcactacc cactggccag ccacccagcc aacaggtctc 1706

tcctcttggg ccctgatttc agagtcctct ttctcactag agactcaatg acagaagaga 1766

ggctgggact tggttgggca tgctgtggtt gctgagggat gaggggagg agagaggtag 1826

gagctggaga tgaagagact gctagaagca gcaggaagcc tgcccttctg ccctctcccc 1886

tccctgcccc tgtgtgagtc ttttagggag ggtgactggg aggtgccccc cgtcccacct 1946

ttttcctgtg ctctaggtgg gctaagtgcc tccctagagg actccatggc tgagaggctc 2006

ctgggcagat ggggtcaagg ctgggccagt cccagatgaa gcctatggga gtcaggaccc 2066

tctccactct ccctctccac tccccttcct gttctcacct ggctgtggct ggccctgtgt 2126

ggggtgggta cactggaaaa caagaaggtt ggagttggtc taggacattg gttttaaatg 2186

acagttctgt gaactggtcc aaggaggttc tgttattaaa gtgatatatg gtcttgaaaa 2246

aaaaaaaaaa aaaaaaaaa 2265

<210> 10

<211> 457

<212> PRT

<213> human

<400> 10

Met Ser Leu Met Leu Asp Asp Gln Pro Pro Met Glu Ala Gln Tyr Ala Glu
-215 -210 -205

Glu Gly Pro Gly Pro Gly Ile Phe Arg Ala Glu Pro Gly Asp Gln Gln His
-200 -195 -190 -185

Pro Ile Ser Gln Ala Val Cys Trp Arg Ser Met Arg Arg Gly Cys Ala Val
-180 -175 -170

Leu Gly Ala Leu Gly Leu Leu Ala Gly Ala Gly Val Gly Ser Trp Leu Leu
-165 -160 -155 -150

Val Leu Tyr Leu Cys Pro Ala Ala Ser Gln Pro Ile Ser Gly Thr Leu Gln
-145 -140 -135

Asp Glu Glu Ile Thr Leu Ser Cys Ser Glu Ala Ser Ala Glu Glu Ala Leu
-130 -125 -120

Leu Pro Ala Leu Pro Lys Thr Val Ser Phe Arg Ile Asn Ser Glu Asp Phe

-115 -110 -105 -100

Leu Leu Glu Ala Gln Val Arg Asp Gln Pro Arg Trp Leu Leu Val Cys His

-95 -90 -85

Glu Gly Trp Ser Pro Ala Leu Gly Leu Gln Ile Cys Trp Ser Leu Gly His

-80 -75 -70 -65

Leu Arg Leu Thr His His Lys Gly Val Asn Leu Thr Asp Ile Lys Leu Asn

-60 -55 -50

Ser Ser Gln Glu Phe Ala Gln Leu Ser Pro Arg Leu Gly Gly Phe Leu Glu

-45 -40 -35

Glu Ala Trp Gln Pro Arg Asn Asn Cys Thr Ser Gly Gln Val Val Ser Leu

-30 -25 -20 -15

Arg Cys Ser Glu Cys Gly Ala Arg Pro Leu Ala Ser Arg Ile Val Gly Gly

-10 -5 -1 1

Gln Ser Val Ala Pro Gly Arg Trp Pro Trp Gln Ala Ser Val Ala Leu Gly

5 10 15 20

Phe Arg His Thr Cys Gly Gly Ser Val Leu Ala Pro Arg Trp Val Val Thr

25 30 35

Ala Ala His Cys Met His Ser Phe Arg Leu Ala Arg Leu Ser Ser Trp Arg

40 45 50 55

Val His Ala Gly Leu Val Ser His Ser Ala Val Arg Pro His Gln Gly Ala

60 65 70

Leu Val Glu Arg Ile Ile Pro His Pro Leu Tyr Ser Ala Gln Asn His Asp

75 80 85

Tyr Asp Val Ala Leu Leu Arg Leu Gln Thr Ala Leu Asn Phe Ser Asp Thr

90 95 100 105

Val Gly Ala Val Cys Leu Pro Ala Lys Glu Gln His Phe Pro Lys Gly Ser
110 115 120
Arg Cys Trp Val Ser Gly Trp Gly His Thr His Pro Ser His Thr Tyr Ser
125 130 135 140
Ser Asp Met Leu Gln Asp Thr Val Val Pro Leu Phe Ser Thr Gln Leu Cys
145 150 155
Asn Ser Ser Cys Val Tyr Ser Gly Ala Leu Thr Pro Arg Met Leu Cys Ala
160 165 170
Gly Tyr Leu Asp Gly Arg Ala Asp Ala Cys Gln Gly Asp Ser Gly Gly Pro
175 180 185 190
Leu Val Cys Pro Asp Gly Asp Thr Trp Arg Leu Val Gly Val Val Ser Trp
195 200 205
Gly Arg Ala Cys Ala Glu Pro Asn His Pro Gly Val Tyr Ala Lys Val Ala
210 215 220 225
Glu Phe Leu Asp Trp Ile His Asp Thr Ala Gln Asp Ser Leu Leu
230 235 240

<210> 11

<211> 99

<212> DNA

<213> Artificial Sequence

<220>

<223> Designed oligonucleotide to construct plasmid pSecTrypHis

<400> 11

aagcttggct agcaacacca tgaatctact cctgatcctt acctttgttg ctgctgctgt 60 tgctgccccc

tttgacgacg atgacaagga tccgaattc 99

<210> 12

<211> 99

<212> DNA

<213> Artificial Sequence

<220>

<223> Designed oligonucleotide to construct plasmid pSecTrypHis

<400> 12

gaattcggat ccttgtcatc gtcgtcaaag ggggcagcaa cagcagcagc aacaaaggta 66 aggatcagga

gtagattcat ggtgttgcta gccaagctt 99

<210> 13

<211> 15

<212> DNA

<213> Artificial Sequence

<220>

<223> Designed oligonucleotide primer to amplify neurosin-encoding sequence

<400> 13

ttggtgcatg gcgga 15

<210> 14

<211> 27

<212> DNA

<213> Artificial Sequence

<220>

<223> Designed oligonucleotide primer to amplify neurosin-encoding sequence

<400> 14

tcctcgagac ttggcctgaa tggtttt 27

<210> 15

<211> 35

<212> DNA

<213> Artificial Sequence

<220>

<223> Designed oligonucleotide primer to amplify a portion of plasmid pSecTrypHis/Neurosin

<400> 15

gcgctagcag atctccatga atctactcct gatcc 35

<210> 16

<211> 29

<212> DNA

<213> Artificial Sequence

<220>

<223> Designed oligonucleotide primer to amplify a portion of plasmid pSecTrypHis/Neurosin

<400> 16

tgaagcttgc catggaccaa cttgtcatc 29

<210> 17

<211> 26

<212> DNA

<213> Artificial Sequence

<220>

<223> Designed oligonucleotide primer to amplify a portion of plasmid pTrypHis

<400> 17

ccaagcttca ccatcaccat caccat 26

<210> 18

<211> 17

<212> DNA

<213> Artificial Sequence

<220>

<223> Designed oligonucleotide primer to amplify a portion of plasmid pTrypSigTag

<400> 18

gcacagtcga ggctgat 17

<210> 19

<211> 17

<212> DNA

<213> Artificial Sequence

<220>

<223> Designed oligonucleotide primer to amplify a portion of plasmid pFBTrypSigTag

<400> 19

caaatgtggt atggctg 17

<210> 20

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Designed oligonucleotide primer to amplify conserved region of serin proteases-encoding sequence

<220>

<221> UNSURE

<222> 9, 12

<223> n is a, c, g or t.

<400> 20

gtgctcacng cngcbcaytg 20

<210> 21

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Designed oligonucleotide primer to amplify conserved region of serin proteases-encoding sequence

<220>

<221> UNSURE

<222> 12, 15

<223> n is a, c, g or t.

<400> 21

ccvctrwsdc cnccnggcga 20

<210> 22

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> Designed oligonucleotide primer designated as mBSSP2.0 for RACE for mBSSP2 (forward)

<400> 22

atggtggaga agatcattcc t 21

<210> 23

<211> 19

<212> DNA

<213> Artificial Sequence

<220>

<223> Designed oligonucleotide primer designated as mBSSP2.1 for RACE for mBSSP2 (forward)

<400> 23

tacagtgccc agaaccatg 19

<210> 24

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Designed oligonucleotide primer designated as mBSSPF4 for RACE for mBSSP2 (forward)

<400> 24

ctcaactctc tgctagaccg 20

<210> 25

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Designed oligonucleotide primer designated as mBSSP2F5 to amplify mature mBSSP2-encoding region (forward)

<400> 25

atagttggcg gccaagctgt 20

<210> 26

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Designed oligonucleotide primer designated as mBSSPF7 to amplify full-length mBSSP2-encoding mRNA (forward)

<400> 26

cccagcagaa cttactgcct 20

<210> 27

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Designed oligonucleotide primer designated as mBSSP2.2 for RACE for mBSSP2 (reverse)

<400> 27

tgttgcagag gtgggtgctg 20

<210> 28

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> Designed oligonucleotide primer designated as mBSSP2R2 for RACE for mBSSP2 (reverse)

<400> 28

taccattgtg tcctgcagtg t 21

<210> 29

<211> 27

<212> DNA

<213> Artificial Sequence

<220>

<223> Designed oligonucleotide primer designated as mBSSP2R5/E to amplify full-length mBSSP2-encoding mRNA (reverse)

<400> 29

tgaattctgc tgcttcttcg gctagcg 27

<210> 30

<211> 18

<212> DNA

<213> Artificial Sequence

<220>

<223> Designed oligonucleotide primer designated as BSSP2SPF to amplify a portion of hBSSP2 (forward)

<400> 30

actgctgccc actgcatg 18

<210> 31

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> Designed oligonucleotide primer designated as BSSP2SPR to amplify a portion of hBSSP2 (reverse)

<400> 31

caggggtccc ccgctgtctc c 21

<210> 32

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Designed oligonucleotide primer designated as hBSSP2F11 for RACE for hBSSP2 (forward)

<400> 32

gctctcaact tctcagacac 20

<210> 33

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Designed oligonucleotide primer designated as hBSSP2R12 for RACE for hBSSP2 (reverse)

<400> 33

actcagctac cttggcgtag 20

<210> 34

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Designed oligonucleotide primer designated as hBSSP2R11 for RACE for hBSSP2 (reverse)

<400> 34

cctggagcat atccgagctg 20

<210> 35

<211> 18

<212> DNA

<213> Artificial Sequence

<220>

<223> Designed oligonucleotide primer designated as hBSSP2F12 to amplify full length hBSSP2 (forward)

<400> 35

gctttacaac agtgctac 18

<210> 36

<211> 28

<212> DNA

<213> Artificial Sequence

<220>

<223> Designed oligonucleotide primer designated as hBSSP2R13/E to amplify full length hBSSP2 (reverse)

<400> 36

tggaattcga ggaaacagca ggactcag 28

<210> 37

<211> 19

<212> DNA

<213> Artificial Sequence

<220>

<223> Designed oligonucleotide primer for RACE for hBSSP2

<400> 37

tactagtcga cgcgtggcc 19

<210> 38

<211> 18

<212> DNA

<213> Artificial Sequence

<220>

<223> Designed oligonucleotide primer designated as hBSSP2F13 to amplify a portion of hBSSP2 (forward)

<400> 38

actgctgccc actgcatg 18

<210> 39

<211> 35

<212> DNA

<213> Artificial Sequence

<220>

<223> Designed oligonucleotide primer designated as FBTrpsigtagF5 to detect hBSSP2

<400> 39

gcgctagcag atctccatga atctactcct gatcc 35

<210> 40

<211> 117

<212> DNA

<213> Artificial Sequence

<220>

<223> Designed oligonucleotide to construct plasmid pTrypHis

<400> 40

aagcttggct agcaacacca tgaatctact cctgatcctt acctttgttg ctgctgctgt 60

tgctgccccc tttcaccatc accatcacca tgacgacgat gacaaggatc cgaattc 117

<210> 41

<211> 117

<212> DNA

<213> Artificial Sequence

<220>

<223> Designed oligonucleotide to construct plasmid pTrypHis

<400> 41

gaattcggat ccttgtcatc gtcgtcatgg tgatggtgat ggtgaaaggg ggcagcaaca 60

gcagcagcaa caaaggtaag gatcaggagt agattcatgg tgttgctagc caagctt 117